Effects of organic and inorganic phosphorus sources on agronomic performance, microbial communities and forage quality of silage corn cultivated under cool climatic conditions

By

Waqas Ali

A thesis submitted to the School of Graduate Studies

In partial fulfillment of the requirements for the degree of

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Approved By:

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Supervisor

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Abstract

Phosphorus (P) is a limiting nutrient for crop growth and yield on more than 30% of the world's arable land, and by some assessments, global resources of mineral P might be depleted by 2050. Additionally, P fertilizer application to soil is predominantly bound to Al^{+3} and Fe^{+2} in acidic soils or with Ca^{+2} in alkaline soils, thereby reducing its availability to agricultural crop. Therefore, P availability in soil rhizosphere and efficient utilization by agricultural crops is vital for economic and environmental reasons. Dairy manure (DM) is an important source of macro and micro nutrients, and organic matter which can affect minerals solubility and plant nutrients availability by improving soil physiochemical properties, enzymes activities and soil microbial communities. To further enhance our understanding about the effects of organic and inorganic P fertilizer sources on growth, forage yield and quality of silage corn, soil biochemical attributes and microbial communities, a field experiment was carried out at Pynn's Brook Research Station (PBRS) for two years (2016 and 2017). Experimental treatments were four P sources: [P_1: manure with high P conc.; P_2: manure with low P conc.; P_3: inorganic P and P_0 (control)] and five silage corn genotypes laid out in Randomized Complete Block Design (RCBD). Agronomic performance (leaf area, chlorophyll contents, photosynthesis, shoot dry weight, root shoot ratio and dry matter yield), rhizosphere soil biochemical attributes (pH, phosphatase activity and soil available P), and microbial community composition and forage quality parameters [(minerals, protein, sugar, fibers, non-fibrous carbohydrates (NFC), total digestible nutrients (TDN) and energy] were measured. Yukon R and DKC26-28RIB showed superior agronomic performance and produced higher dry matter yield

compared to other genotypes. High P manure application increased the dry matter yield by 28% and 33%, acid phosphatase activity by 29% and 44%, soil available P by 60% and 39% compared to control in 2016 and 2017 respectively. High P manure application significantly increased active Gram negative (G-) bacteria, active fungi, total active bacterial phospholipids fatty acids (PLFAs), and active soil microbial biomass (total amount of PLFAs) compared to inorganic P and control treatments. Yukon R and DKC26-28RIB showed higher active fungal biomass, bacteria as well as total microbial activities in their root rhizospheres regardless of P sources compared to the other genotypes evaluated. Pearson correlation analysis demonstrated a positive and strong relationship between the active microbial community structure and the agronomic performance of the silage corn genotypes amended with P sources. The results indicate that DM application significantly enhanced agronomic performance, soil biochemical attributes and microbial communities compared to inorganic P fertilizer. Silage corn genotypes had significant effects on forage quality indices. Yukon-R and DKC26-28RIB showed superior agronomic performance and produced higher forage production of 16.43 and 15.47 Mg ha^{-1} respectively. Contrarily, A4177G3RIB produced higher minerals, protein, total digestible nutrients (TDN), net energy for maintenance (NEM) and net energy for gain (NEG) compared to other genotypes, and proved high quality forage. However, it was statistically at par with DKC26-28RIB in producing minerals and ranked 2^{nd} in protein synthesis. Yukon R was also statistically at par with A4177G3RIB genotype in few quality indices and produced similar TDN, NEM and NEG. Overall, Yukon–R produced high forage and was either at par or ranked at 2^{nd} position in most of the quality forage indicators.

Phosphorus sources had significant effects on crude protein (CP), available protein (AP), sugars, and non-fibrous carbohydrates (NFC) contents of silage corn but not significantly higher with each other. Inorganic P fertilizer source enhanced CP, AP, simple sugars and NFC contents but statistically at par either with manure with high or low P. Phosphorus sources had non-significant effects on energy parameters, which are important indicator of high forage quality, however NEL, NEM and NEG values were slightly higher with DM application compared to inorganic P and control. Dairy manure application significantly improve the agronomic performance, silage corn quality, soil physiochemical properties and soil microbial communities. Future studies needed to investigate the effects of organic and inorganic P amendments on soil phenolics and root exudates on P availability and microbial community structure.

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Waqas Ali

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List of Abbreviations

% - Percentage

@ - at the rate

µg/mL – Microgram per milliliter

µl – Microliter

µmol photon m^{-2} s^{-1} – Micromole photon per square meter per second

ADF – Acid detergent fibers

Al^{+3} – Aluminum

AMF – Arbuscular mycorrhizal fungi

ANOVA – Analysis of variance

AP – Available protein

AP-ase – Acid phosphatase activity

ATP – Adenosine triphosphate

BAME – Bacterial acid methyl ester

C – Carbon

C: N – Carbon: Nitrogen ratio

Ca^{+2} – Calcium

$CaCl_2$ – Calcium chloride

CHU – Corn heating unit

cm^2 – Square centimeter

CO_2 – Carbon dioxide

$COOH^-$ - Carboxyl group

CP – Crude protein

DAS – Days after sowing

DCP – Di-calcium phosphate

DM – Dairy Manure

DMY – Dry matter yield

DNA – Deoxyribonucleic acid

F_1 – First component

F_2 – Second component

FAME – Fatty acid methyl ester

FAO – Food and Agriculture organization

Fe^{+2} – Iron

g – Gram

G^- - Gram negative bacteria

G^+ - Gram positive bacteria

G^+/G^- - Gram positive bacteria / Gram negative bacteria ratio

GABA – Gamma aminobutyric acid

GC-FID – Gas chromatography – Flame ionization detection

GxExM – Genotype x Environment x Management

H^+ - Hydrogen ion

$H2PO_4^{-1}$ – Primary orthophosphate

ha - Hectare

HAP – Hydroxyapatite

$HPO4^{-2}$ – Secondary orthophosphate

IP – Inorganic phosphorus

K – Potassium

L – Liter

LA – leaf area

LED – Light emitting diode

MAP – Mono-ammonium phosphate

Mcal Kg^{-1} – Mega-calorie per kilogram)

MCP – Mono-calcium phosphate

Mg – Magnesium

Mg ha^{-1} – Mega gram per hectare

mL – Milliliter

mm – millimeter

N – Nitrogen

NADPH – Nicotinamide adenine dinucleotide phosphate oxidase)

NaOH – Sodium hydroxide

NDF – Neutral detergent fibers

NEG – Net energy for gain

NEL – Net energy for lactation

NEM – net energy for maintenance

NFC – Non-fibrous carbohydrates

NIR – Near Infrared reflectance analysis

NIST – National Institute of Standard and Technology

NL – Newfoundland and Labrador

nm – Nanometer

$nmolg^{-1}$ – Nano-mole per gram

NRC – National Research Council

NUE – Nitrogen use efficiency

^{o}C – Degree centigrade

OH^{-} - Hydroxyl group

ON – Ontario

OP – Organic Phosphorus

P – Phosphorus

p – Probability or level of significance

P_0 or DM_0 - Control

P_1 or DM_1 – Dairy manure with high phosphorus concentration

P_2 or DM_2 – Dairy manure with low phosphorus concentration

P_3 - Inorganic phosphorus

PAR – Photosynthetically active radiation

PAST – Paleontological statistics

PBRS - Pynn's Brook Research Station

PCA – Principal component analysis

PDE – Phosphodisterase

PH – Plant height

pH – Power of hydrogen ion

P_i – Inorganic P

PLFAs – Phospholipids fatty acids

PNP – P-nitro phosphate

ppm – Parts per million

PSB – Phosphate solubilizing bacteria

PSF – Phosphate solubilizing fungi

PUE – Phosphorus use efficiency

Q_1 – First quadrant

Q_2 – Second quadrant

Q_3 – Third quadrant

Q_4 – Four quadrant

RCBD – Randomized complete block design

R-CHO – Aldehyde group

RIB – Refuge is in the bag

RNA – Ribonucleic acid

rpm – Revolutions per minute

RR – Round up ready

RR2 – Resistant gene to roundup^{R} and factor 540^{R}

RuBP – Ribulose 1, 5-bisphosphate

SAP – Soil available phosphorus

Si – Silica

SOC – Soil organic carbon

SPAD – Soil plant analysis development

SPE – Solid phase extraction

SS – Simple sugars

TDN – Total digestible nutrients

TMSH – Trimethyl sulfonium hydroxide

USA – United states of America

USDA – NASS – United States Department of Agriculture - National Agriculture Statistics

v/v – Volume over volume

ΣB-PLFAs – Total bacterial phospholipid fatty acids

ΣPLFAs – Total phospholipid fatty acids

Chapter 1

1. General Introduction and Literature Review

1.1. Corn

Corn (*Zea mays* L.) is a member of Poaceae family and ranked third in cereal production after wheat and rice. It is a tropical crop but successfully grown in subtropical and temperate climatic zones (Tagne et al., 2008). It is widely grown as cereal production across the globe with approximately 10,000 million tons per year whereas, Canada produces 14.10 million tons (United State Department of Agriculture, 2018). Corn plant as a whole is important forage for dairy and beef animals. It is considered as the most suitable silage crop as compared to other cereals due to faster growth, higher yield potential, higher palatability, energy contents, protein contents, sugar and water soluble carbohydrate which are most important in the preservation of silage material (Amin, 2011; Keady et al., 2008). Good quality corn silage contains 28-32% dry matter yield, 28-32% starch and 7-9% crude protein (Keady et al., 2008; Kwabiah et al., 2003; Ullah et al., 2015) Addition of corn in silage based diets of dairy cows increases feed intake, yield and protein content of milk (Keady et al., 2008). As a result, significant acreage in silage corn across the world has been noticed over the past few decades. It has become a major feed component in the ration of dairy cows under most dietary regimes (Keady et al., 2008). Increasing demand of silage corn due to rapid expansion of dairy industry, it is important to determine the forage production potential and quality indices of silage corn under different nutrient management regimes.

Presently, Newfoundland and Labrador (NL), dairy and livestock industry faces challenges of insufficient silage/forage production and it has to depend on substantial imports from the mainland Canada and other countries. In 2011, area under silage corn was 324.96 ha which decreased to 205.98 ha in 2016 (Statistics Canada, 2017). One of the reason for this decline is unique NL weather and soil conditions which limits crop growth and forage production (Kwabiah, 2003). For example, silage corn cultivation in NL is limited by low corn heating unit (CHU), and typically shallow, coarse-sandy texture and high acidic soil (pH 4 to 4.5) (Sauer et al., 2007). Furthermore, macro and micronutrients availability significantly affect the dry matter yield of silage corn (Leytem et al., 2011), especially under podzol soils where low P availability due to metal complexation has been shown to be a major constraint under field conditions (Leytem et al., 2011).

These challenges can be reduced by generating scientific based information about suitable short growing season silage corn hybrids, nutrient management practices and crop husbandry required to exploit the production potential of silage corn under cool climatic conditions of NL. Growing of tested silage/forage/fodder genotypes might bridge the gap between production and consumption. On the other hand, dairy farm operations across NL produce a large quantity of manure, which is an inexpensive and abundant source of plant nutrients. Silage corn has emerged as an important feed-stuff in the province. It is known as a high energy crop, with additional benefits such as the potential to improve the leg and hoof soundness and increase heat detection. Therefore, present study was conducted to elucidate the influence of organic and inorganic P source on agronomic performance, biochemical, and microbial community structure and quality indices of silage corn.

1.2. Phosphorus in Agricultural ecosystems

Phosphorus (P) is the 11th most abundant element in the earth crust and reach to 1200 mg/kg in soil. However, most of the soils contains 200-800 mg/kg, while older soils contains less P relative to younger soils (Ijaz et al., 2017). Phosphorus limitations are much more severe in agricultural ecosystems, because it is removed from soil by harvesting crops and only small quantity of P returned back to ecosystem by incorporating crop residues, animal manures and chemical fertilizers. Consequently, extreme P deficiencies are quite common where no external source of P is applied to soil and such conditions are widespread all around the globe (Liu et al., 2013). Excessive or little P application led to severe and negative impacts on environment by causing by land degradation under P deficient conditions and eutrophication under excessive application (Shen et al., 2011).

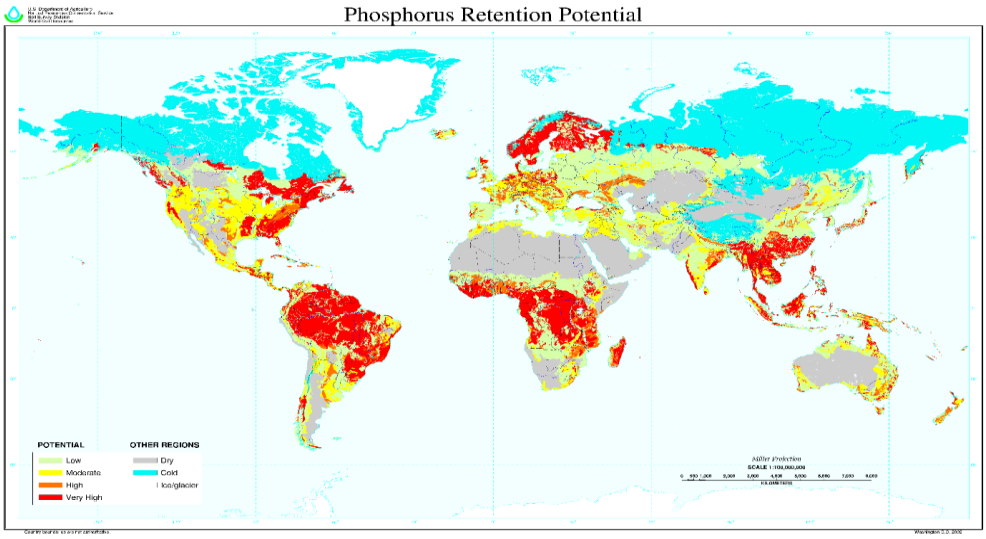

Figure 1.2.1: Global phosphorus retention map (USDA NRCS, 2012)

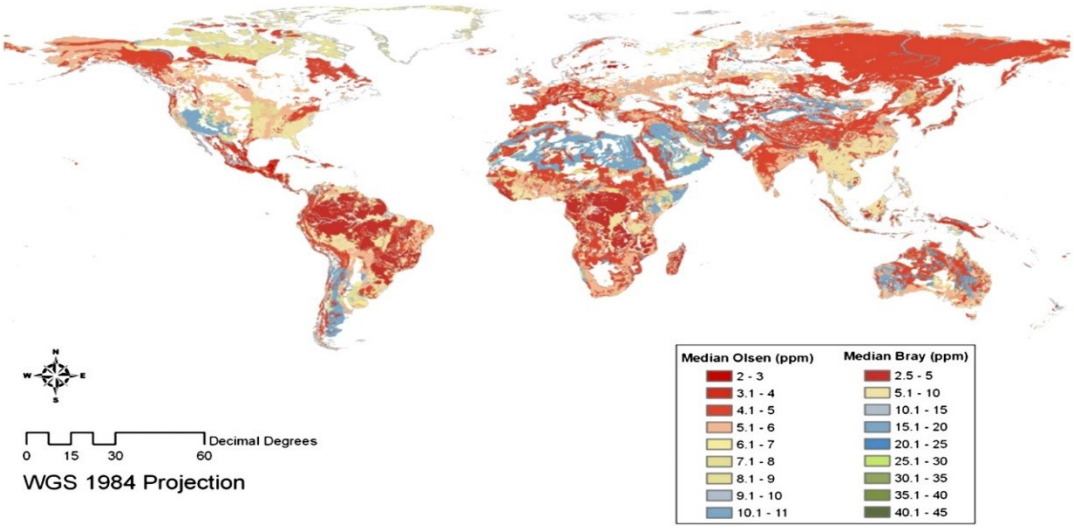

Figure 1.2.2: Global phosphorus availability map. The red and light-gray colors, showing suboptimal P availability for the plant growth, shows the importance of P availability as a most limiting nutrient to plant productivity in terrestrial environment (Jaramillo-Velastegui, 2011).

The global crop productivity has strong relationship with soil fertility and most of the world soils are P deficient hence leading to lower crop productivity, however P deficiency is more often found in old weathered soils (Jaramillo-Velastegui, 2011) (Figure 1.2.1 & Figure 1.2.2). Consequently, P deficient soils are supplemented with external P application through chemical fertilizers or other organic sources (compost, pig slurries, sludge, digestate, manures, and crop residues etc.). Phosphorus cycle is the biogeochemical cycle that describes the movement of P through land, water and living things (Shen et al., 2011) and major process involved the uptake of P by plants, recycling (the return of plant and animal residue), biological processes includes mineralization and fixation, and then solubilization of fixed P mediated by bacterial communities (Chen et al., 2006; Wei et al.,

2017), enzymes (Waldrip et al., 2012), and arbuscular mycorrhizal fungi (Smith and Read, 2008).

1.3. Fate of organic and inorganic P in soil

Chemical forms of soil P include organic and inorganic P (IP), and these forms differ in their behaviour and fate in soil (Turner et al., 2007). Organic phosphorus (OP) generally accounts for 30-65% of total P in soils, and average concentration range between 50-500 mg/kg of soil (Harrison, 1987). It is present in stabilized forms such as inositol phosphates, phosphonates, and active forms including orthophosphate diesters, labile orthophosphate monoesters, and organic polyphosphates (Turner et al., 2002). Organic phosphorus transformed into plant available P forms such as primary orthophosphate ($H_2PO_4^{-1}$) and secondary orthophosphate (HPO_4^{-2}) by mineralization processes mediated by soil microorganisms and phosphatase enzyme released by plant roots (Waldrip et al., 2012). Primary orthophosphate mostly dominant in acidic conditions and secondary orthophosphate in alkaline conditions, whereas at neutral pH both forms exists in equal amount (Shen et al., 2011). Mineralization process is highly influenced by soil physiochemical properties i.e. soil moisture, temperature, surface physiochemical properties, microorganisms and enzymes, i.e. soil moisture, temperature, surface physiochemical properties, pH and acid phosphatase activity (Shen et al., 2011). Organic P transformation has a great influence on the overall bioavailability of soil P (Turner et al., 2007), and plant available P is very complex and needs to be systematically evaluated,

because it is highly related with P dynamics and transformation among various pools (Figure 1.4.1).

Inorganic P usually accounts for 35 – 70% of total P in soil (Harrison, 1987). Phosphorus exist in two mineral forms; Primary P minerals including apatites, strangite and variscite are very stable and available P is released by chemical weathering very slowly from these minerals to meet crop demands, while direct application of rock phosphate (i.e. apatite) has proved relatively efficient mineral P source for crop growth in acidic conditions. Whereas secondary P minerals include calcium (Ca^{+2}), iron (Fe^{+2}) and aluminum (Al^{+3}) phosphates vary in dissolution depending upon size of mineral particles and soil pH (Oelkers and Valsami-jones, 2008). With increasing soil pH from acidic to neutral, solubility of iron and aluminum phosphates increases, but solubility of calcium phosphates decreases except for pH values above 8 (Hinsinger, 2001). Desorption reaction helps to release absorbed P from Al/Fe oxides and all these P forms are present in complex equilibria with each other, from very stable, sparingly available, to plant available pools such as labile and solution P (Figure 1.4.1) (Shen et al., 2011).

Phosphorus availability to plants is mainly dependent upon soil pH, Al, Fe, Ca, decomposition of organic matter and activities of microorganisms (Arai and Sparks, 2007; Shen et al., 2011; Wei et al., 2017). In acidic soils, IP usually forms chelates with Al and Fe oxides and hydroxides, such as gibbsite, hematite, and goethite (Parfit, 1989). The non-protonated and protonated ligands surface complexes may coexist at pH 4-9, while protonated ligand inner sphere complex is predominant under acidic conditions (Arai and

Sparks, 2007). In neutral to calcareous soils, precipitation reaction is dominated and P is adsorbed on the surface of calcium carbonate (Devau et al., 2010). Phosphate react with Ca and transformed into plant available phosphate form such as dicalcium phosphate (DCP), which can be changed to more stable forms such as octocalcium phosphate and hydroxyapatite (HAP) which are less available to plants at alkaline pH (Arai and Sparks, 2007).

1.4. Phosphorus transformation in soil following manure and inorganic P sources

Dairy farming is main agricultural sector in Canada with 945000 dairy cows present on 10951 farms across the Canada (Statistics Canada and Canadian Dairy Commission, 2017). Dairy and poultry farming is a large segment of the NL economy, and a total of 510 agricultural farms including 32 dairy farms, 5600 cows and 2200 heifers are present in the province (Statistics Canada, 2017; Statistics Canada and Canadian Dairy Commission, 2017). Therefore, dairy manure (DM) available in bulk quantities and is applied to increase soil fertility status for growing crops. DM is historically known as a rich source of nitrogen (N), phosphorus (P), and potassium (K) and micronutrients for plant growth and also improves aggregation and soil structure for better aeration, supplies organic matter, improves soil quality and maintains or increase soil pH in acidic soils (Dong et al., 2012; Hirzel and Walter, 2008; Yan et al., 1996). DM application improve plant physiological characteristics and dry matter yield by improving PUE and nitrogen use efficiency (NUE) (Bernier et al., 2014). An efficient utilization of DM is important to improve the sustainability of animals and crop production systems. Crop growth and dry matter yield

is a measure of plant photosynthesis efficiency (Iqbal et al., 2014), which is mainly influenced by balance nutrient availability (Ibeawuchi et al., 2007) and environmental factors (Amin, 2011).

Dairy farmers, vegetable growers and organic growers across the NL use DM as a nutrient source for crop production, but excessive application of manure build up high amount of P in the soils and cause runoff during high rainfall periods which pollute water bodies by eutrophication (Carpenter, 2005). Therefore, management of land application of DM is important to maximize the nutrients use efficiency and organic matter, while minimizing its impact on the environment. Total P content in manure is variable and generally 70% of the total P in manure is labile (Shen et al., 2011). DM contains 50-90% of IP (Duo et al., 2000), and large amount of OP such as phospholipids and nucleic acids (Turner and Leytem, 2004), which can be mineralized to available P in soil. During the mineralization process organic acids produced from humic substances can dissolve calcium phosphate, and especially for citrate, which have ability to efficiently weaken the nanoparticle stability of hydroxyapatite (HAP), by controlling the free Ca availability and nucleation rate (Martins et al., 2008). Humic acids produced from manure mineralization contains large numbers of negative charges, carboxyl (COO^-) and hydroxyl (OH^-) groups, which strongly compete with inorganic P for adsorption sites (Shen et al., 2011).

Modern terrestrial P cycle is dominated by agriculture and anthropogenic activities (Oelkers and Valsami-jones, 2008), and concentration of soil available P seldom increases 10 μM (Bieleski, 1973), which is less than plant tissues concentration range (between 5 to

20 mM inorganic P (Raghothama, 1999). Due to low concentration and poor mobility of plant available P in soils, IP fertilizers application is required to obtain optimum growth and yield. Mono-calcium and mono-potassium phosphate are two major forms of inorganic phosphate fertilizers. Mono-calcium phosphate (MCP) fertilizer in soil undergoes a wetting process, produces large quantity of protons, phosphate, di-calcium phosphate (DCP), and eventually forms an IP saturated patch, this patch forms three reaction zones including direct reaction, precipitation reaction, and adsorption reaction zones (Benbi and Gilkes, 1987). Direct reaction zone in soil is very acidic and pH ranges between 1.0-1.6, resulting in increased mobilization of metal ions, which react with high concentrations of IP in the zone, thus causing further IP precipitation. In alkaline soils, MCP and DCP forms new complexes and gradually transformed into more stable forms of Ca-phosphates such as octacalcium phosphate or apatite and P availability is significantly reduced (Shen et al., 2011). Thus, to improve the nutrient utilization in cropping systems and avoiding P losses, a comprehensive understanding of the influences of organic and IP amendments on agronomic performance, enzymes, available P and microbial communities is necessary.

1.5. Role of root architecture and morphological traits in P uptake

Root architecture refers to the spatial configuration of plant root systems, whereas root morphological traits includes i.e. root surface area, number of roots, root length and volume (Liu et al., 2018). Crops responds to P deficiency by modulating their root architecture including root morphology, topology and distribution patterns (Fernandez and Rubio, 2015). Phosphorus deficiency increase root/shoot ratio, root branching, elongation,

top foraging and cluster root hairs (Lynch and Brown, 2008). Phosphorus starvation causes reduction in primary root growth, increased length and density of lateral root hairs in corn and many other plant species (Lynch and Brown, 2008).

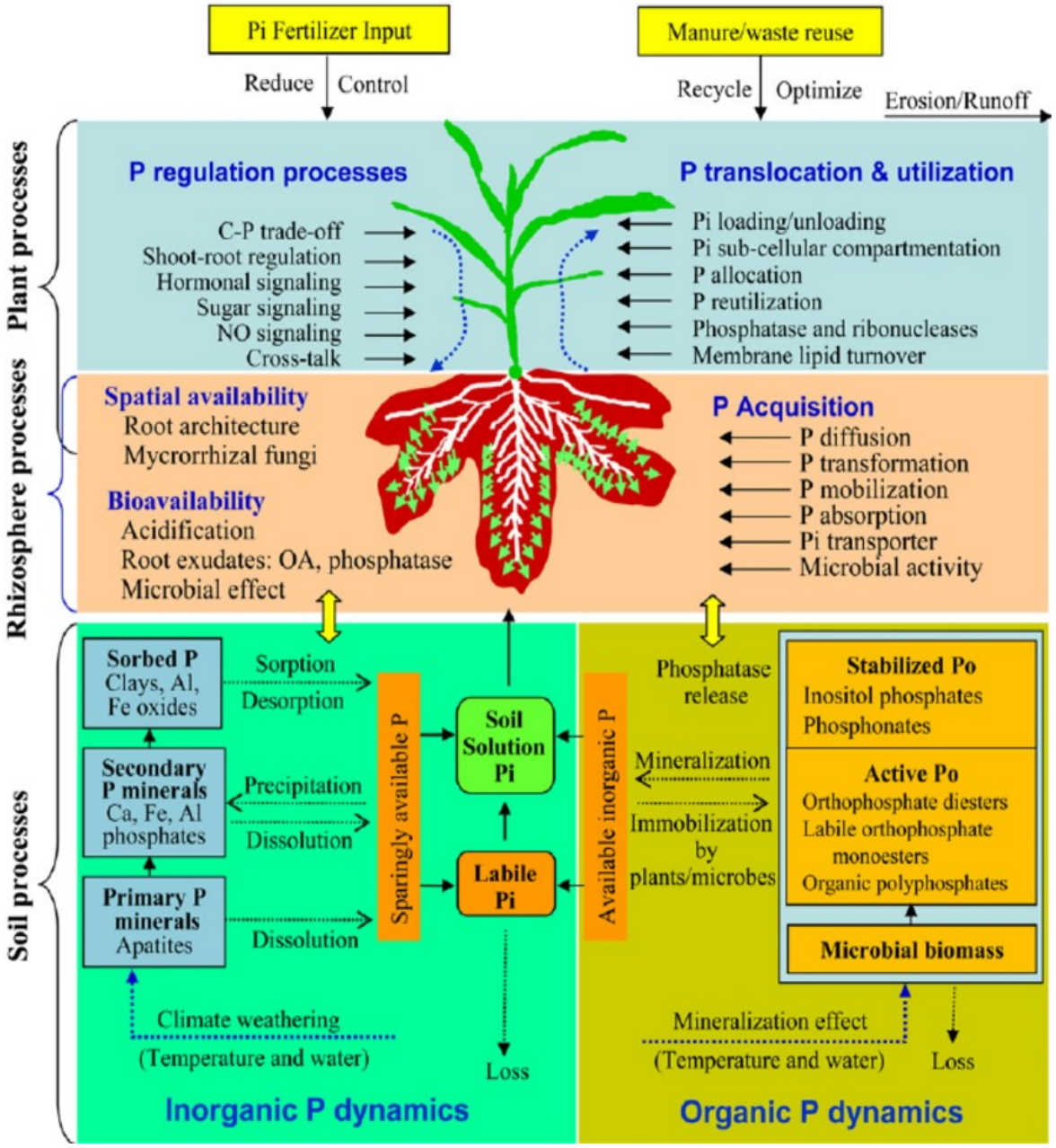

Figure 1.4.1: Phosphorus dynamics in the soil/rhizosphere-plant continuum (Shen et al., 2011)

Under P starvation, adaptive changes in corn root architecture take place, like changes in carbohydrates distribution between roots and shoots (Wissuwa et al., 2005). Phosphorus deficiency in soil induced cluster root formation in white lupin (Vance et al., 2003) but cluster roots distribution shifted from the P deficient root zone to P rich or organic matter rich patches to increase the intensity of soil foraging (Shen et al., 2013; Vance et al., 2003). Inorganic P (IP) fertilizers application significantly improve root growth and stimulate root proliferation (Shen et al., 2013). Furthermore, IP fertilizers application at early growth stages is known to be an effective strategy for stimulating corn root development and establishment of good root architecture and increasing yields, particularly in the early crop growth period at low temperature (Shen et al., 2013). DM application significantly improved root length density compared to control (no P addition) (Zhihui et al., 2016). Corn genotypes exhibited significant difference in root architecture and morphological traits due to difference in genetic potential (Szoboszlay et al., 2015). A larger root system can be a beneficial trait to scavenge more water and nutrients under stress conditions.

1.6. Role of root exudates in P uptake under different P sources

Plants roots exude organic compounds such as mucilage, organic acids, acid phosphatase and some specific signaling substances, which are key drivers of various rhizosphere processes (Shen et al., 2011). Low mobility and solubility of P in soil restricts its availability to plants (Shen et al., 2011). Root secretes mainly protons to acidify the rhizosphere, carboxylate exudation to mobilize sparingly available P by chelation and ligand exchange and secretion of phosphatase to mobilize organic P by hydrolysis (Zhang

et al., 2010). Roots decrease 2-3 units of pH in soil rhizosphere zone compared to bulk soil due to excessive uptake of cations over anions, led to produce protons by plant roots cause acidification, and exudation of citrate, malate and oxalate greatly increase P acquisition through chelation and ligand exchange (Shen et al., 2011). Changes in rhizosphere pH depends upon soil buffering capacity, microbial activities and plant genotypes (Li et al., 2014). In acidic soils or near neutral soils manure can maintain or increase soil pH through liming effect because they contain some calcium carbonate, which originates in the animal diet (Moore and Edwards, 2007). Earlier studies also reported that manure application increased soil pH (Dong et al., 2012).

Corn crop under P deficient conditions secretes mainly primary metabolites in higher concentration such as GABA (Gamma aminobutyric acid), and several sugars such as inositol erythritol, ribitol, fructose, glucose and arabinose in comparison to nutrient replete maize plants (Carvalhais et al., 2013, 2011). GABA secretions by plants linked with signaling in a number of abiotic stressor response (Kinnersley and Turano, 2000), which may act as a signaling compound when maize is P starved. Bacterial communities showed chemotaxis response to sugars (Thoelke et al., 1990). Higher exudation of sugars under P starvation has been linked with a decrease in phospholipids levels and a high permeability of the cell membrane. Sugar secretions in the rhizosphere zone may stimulate germination and growth of mycorrhizal fungi, which are known to improve P acquisition (Graham et al., 1981). Phosphorus starved plants secrete less secondary metabolites that are toxic to bacteria compare to plants grown under optimum conditions. This may occur to attract specific bacterial communities that can improve acquisition of the specific deficient

nutrient. For instance Bacillus amyloliquefaciens promote the growth of P limited corn seedlings in the presence of phytate (Idriss et al., 2002).

Krebs cycle intermediates such as citric, malic, succinic fumaric, and aconitic acids are root exudates secreted by many plant species (Dakora and Phillips, 2002), these acids plays an important role in P acquisition. Phosphorus deficiency led to increase 82% increase in citrate exudation which may help to increase P availability by forming ligand exchange (Lipton et al., 1987). However, different plant species respond differently to P deficiency, rape typically release malic acid near its root tips or at sites in contact with insoluble rock phosphate, whereas mustard dose not secrete organic acids (Hoffland et al., 1992), possibly due to problems associated with synthesis and transport across membranes. Barley showed non-significant difference in citrate exudation under low and sufficient P, whereas malic acid showed an increase in malic acid exudation under P deficient conditions. Different plant species and genotypes exude different types and quantity of organic acids under P starvation and sufficient conditions (Wang et al., 2015)

Approximately 80-90% of soil processes such as decomposition and transformation of nutrients from organic compounds occur through biochemical reactions mediated by enzymes (Acosta-Martinez and Waldrip, 2014). Phosphatase enzyme is an important component of P cycle and it is the most important indicator of soil quality and microbial activity. Phosphatase enzyme hydrolyze OP into plant available form such as ($H_2PO_4^{-1}$ and $H_2PO_4^{-2}$) (Waldrip et al., 2012). Plants can secrete acid phosphatase to hydrolyze OP and acid phosphatase activity can be greatly altered by the availability of substrate, soil

microorganisms, pH and soil physio-chemical properties (George et al., 2005). Phosphatase enzymes exist in five major groups in soils: (1) phosphoric monoester hydrolases (phosphomonoesterases), (2) phosphoric diester hydrolases (phosphodiesterases), (3) phosphoric triester hydrolases (4) polyphosphate hydrolases and (5) enzyme acting on P-N bonds (Waldrip and Acosta-Martinez, 2014). Organic P mineralization started by phosphodiesterase (PDE) hydrolyze phosphodiesters such as phospholipids and nucleic acids to form simpler phosphomonoesters and finally converted to plant available P (Figure 1.6.1) c. Phosphomonoesterase release free phosphate from phosphomonoesters such as inositol phosphate, β-glycerophosphate, phenylphosphate, sugar phosphates, adenosine phosphates, β-napthyl phosphate, and p-nitrophenyl phosphate (He et al., 2004). Among the phosphatase enzymes the most investigated soil phosphomonoesterases are classified according to soil pH such as acid phosphatase and alkaline phosphatase (Waldrip et al., 2012).

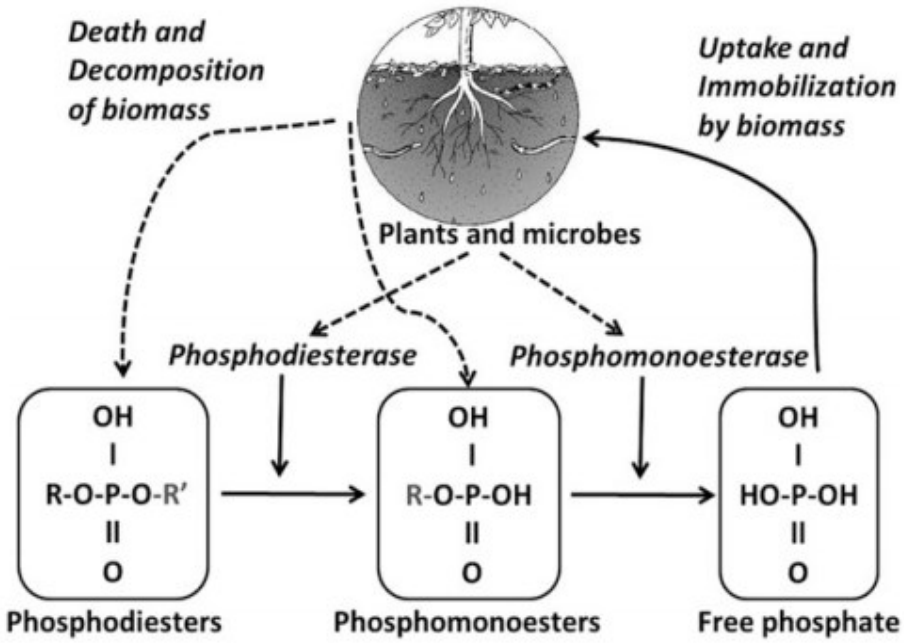

Figure 1.6.1: Conceptual model of the hydrolysis of organic P in to free phosphate mediated by PDE and phoshomonoesterse (Waldrip and Acosta-Martinez, 2014)

Bacteria, fungi and plant roots produce extracellular phosphatase enzyme. Plants can produce only acid phosphatase enzyme, therefore extracellular alkaline phosphatase is strongly linked to soil microorganisms (George et al., 2002). Earlier studies reported that plant species, genotypes, plant growth stages, soil pH, temperature, moisture, SOM and the presence of C, N, P and other nutrients required by microorganisms affect the process of organic P mineralization as well as phosphatase activity (Parham et al., 2002). Dairy manure and organic fertilizers application significantly increase soil phosphatase activity and plant available P in soil (Colvan et al., 2001; Waldrip et al., 2012). Inorganic P fertilizers application significantly reduced the acid phosphatase activity, because plants

and microorganisms easily draw the P form readily available sources and do not have to mineralize organic P (Spohn and Kuzyakov, 2013).

1.7. Role of phosphorus in crop growth and development

Phosphorus (P) is an important macronutrient for plant growth, but uptake from soil can be difficult and limiting factor for optimum crop production (Smit et al., 2009). Phosphorus is a component of nucleic acids and cellular membranes, and important for several metabolic processes and signaling pathway of proteins (protein kinase) (Vance et al., 2003). It plays an important role in cell division being a constituent element of nucleoproteins which are involved in the cell reproduction processes and essential for vegetative and reproductive development (Azeem et al., 2018). It is also a component of nucleotides used in plant energy metabolism such as adenosine triphosphate (ATP), deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Phosphorus deficiency include stunted growth in young plants and a dark green coloration of the leaves, which may be malformed and contain small spots of dead tissues called necrotic spots (Chen et al., 2014).

Optimizing PUE in cereal crops is an important task for agricultural sustainability and global food security. Phosphorus deficiency can severely reduce leaf area and ultimately the amount of solar intercepted radiation and photosynthesis (Plenet et al., 2000). Thus, reduction in leaf area and photosynthesis may contribute to the reduction in final biomass production and nutritional value. Dry matter yield is highly dependent on plant photosynthesis efficiency and sink capacity to accumulate photosynthates from the plant leaves (Warraich et al., 2002). The optimum supply of N, P, K and other

micronutrients increase dry matter production via increasing leaf area production and photosynthesis rate. Phosphorus deficiency in corn severely reduce leaf growth and ultimately the amount of solar intercepted radiation, photosynthesis and dry matter yield (Plenet et al., 2000). Phosphorus deficiency decrease 67 % mean leaf area, and 43 % leaf emergence (Fredeen et al., 1989). Decrease in leaf area under P deficiency occurs due to an insufficiency of phosphate for the expansion of epidermal cells. Because leaf epidermal cell expansion appears to be a critical process in the expansion of the leaf (Waldron and Terry, 1987) and phosphate concentrations in the upper epidermis are rapidly reduced with decrease in P supply to the leaf (Treeby et al., 1987).

Chlorophyll contents are important in energy harvesting reaction that can be used to assimilate carbon dioxide. Higher concentration of chlorophyll contents and ribulose 1,5-bisphosphate (RuBP) had shown increased photosynthetic rate in plants (Reich et al., 1994). Leaf phosphorus and chlorophyll contents showed positive relationship with each other (Ryser et al., 1997). Chlorophyll is the part of chloroplast, and it is predominantly consuming inorganic phosphate (Pi) (Giersch and Robinson, 1987). Deficiency of P supply to chloroplast inhibits the process of photosynthesis (Dietz and Foyer, 1986). DM and other organic fertilizers provide essential nutrients for plant growth and have a positive effect on the formation of chlorophyll and chloroplast. Organic acids play an important role in the supply of Mg which have an important role in the formation of the chlorophyll molecule (Hasan et al., 2014).

Biomass production is the outcome of interception of photosynthetically active radiation (PAR) by plant leaves and the ability of plants canopy architecture to transform the intercepted PAR in to biomass production (Plenet et al., 2000; Portes and Melo, 2014). Phosphorus deficiency reduce the export of triose-P from the chloroplast to the cytosol via the Pi translocator, which may lead to buildup of starch and sucrose, and decrease the rate of photosynthesis (Giersch and Robinson, 1987). Phosphorus deficient plants shows reduction in photosynthesis due to diminishing of RuBP generation rather than due to limitations in the supply of ATP and NADPH (nicotinamide adenine dinucleotide phosphate oxide) to the Calvin cycle (Fredeen et al., 1989). Plants can sustain photosynthetic rate under low P supply by increasing synthesis of P – free carbon compounds, e.g. starch, sucrose and increased phosphatase activity and decreased levels of P containing molecules e.g. sugar phosphates and adenylate (Rao et al., 1989). By resupplying the phosphorus to moderate P deficient plants, it can restore the photosynthetic metabolism to control levels within a few hours, that sugar phosphate especially RuBP levels increase as starch and sucrose level decrease (Rao and Terry, 1995).

1.8. Soil microbial communities

Soil microorganisms play an important role in stimulating soil organic matter decomposition, improves nutrient cycling, soil fertility, soil quality and mitigating soil pollution (Khalil et al., 2013). Soil microbial communities can influence soil physiochemical properties, climatic and biological factors and therefore are closely related to crop productivity (Zhang et al., 2015b). Soil and rhizosphere microorganisms such as P

solubilizing bacteria (PSB) and fungi (PSF) can increase the P availability by increasing P solubilization, and accounts for 1-50% in P solubilization potential (Chen et al., 2006). Manure and chemical fertilizer application is an important nutrient management practice that affects soil quality, health and sustainability of agricultural production systems (Wei et al., 2017). Excessive use of chemical fertilizers commonly reduce soil organic carbon (SOC) consequently decline soil quality, health and crop productivity. To maintain and improve SOC, management practices such as DM application, no tillage, crop rotation and crop residue incorporation have been very productive in improving soil quality and health in different cropping systems (Huang et al., 2015; Wei et al., 2017; Yan et al., 2013). Therefore, it can increase plant nutrients availability and enhance soil microbial biomass, activity and diversity (Wei et al., 2017). Different fertilization regimes affect soil microbial communities, as bacteria is mostly adapted to high available C and rich nutrient conditions, whereas fungi seems to be more capable to use recalcitrant C sources (Li et al., 2018; Liu et al., 2017). The appropriate utilization of organic and inorganic amendment, for instance, DM and other organic fertilizer application could be a useful approach in restoring SOC, soil fertility and sustainability (Sharpe et al., 2004), and therefore can increase plant nutrients availability and enhance soil microbial biomass, activity and diversity (Mandal et al., 2007; Wang et al., 2008). However, soil microbial communities benefiting from DM application differ with soil type and physiochemical properties, quantity and quality of manure, experimental duration and many other factors (Cederlund et al., 2014; Wei et al., 2017).

Chemical fertilizers applications can produce positive or negative effect on soil microbial biomass and activity (Geisseler and Scow, 2014), while the effects on microbial community structure and diversity is still under debate (Zhang et al., 2015a). Most studies focused on the individual effects rather than combination effects of specific organic amendments on soil physicochemical properties. The biological properties of soils under different organic and inorganic fertilizer application have been the focus of recent studies (Chen et al., 2011; Chu et al., 2007; He et al., 2007; Jiang et al., 2014). Until present, our knowledge about the effects of different organic and inorganic P sources on soil microbial community and consequently their role in P mineralization and availability to crop plants in podzolic soils remains limited and needs to be investigated.

Phospholipid fatty acids analysis (PLFAs) profiles reveals the structural characteristics of the microbial community and also provides estimate of the abundance and diversity about various microbial groups (Ai et al., 2012). The microbial community, size and activity showed by PLFAs vary with different fertilizer managements, and therefore affects soil fertility and productivity (Ai et al., 2012). Diversity and abundance in soil microbial community was significantly increased due to P rich environment resulted from long term P fertilization research trial, in comparison to control (Tan et al., 2013). Inorganic P fertilization not only influenced the soil microbial abundances but also altered bacterial composition (Liu et al., 2013). Wei et al. (2017) reported that Gram negative (G^-) bacteria proliferate and grow faster soon after the addition of organic materials, and then decrease and also facilitate the growth of other slow growing microorganisms such as Gram positive (G^+) bacteria or fungi. Dairy manure application significantly increased G^-

bacterial biomass relative to G^+ bacterial biomass due to higher availability of soluble carbon over a long part of the year under DM than in the inorganic fertilization system (Peacock et al., 2001). Moreover, higher proportion of G^- bacteria usually occurs following a shift from nutrient deficient to nutrient rich conditions, and this pattern was observed in soil amended with P fertilizers (Tan et al., 2013).

Fungi play an important role in C and nutrient cycling in agricultural ecosystems and is sensitive to fertilizers application (Li et al., 2018). Manure application significantly increased fungal population due to increase in organic C contents which could serve as major source of energy whereas, small increase in labile organic C under inorganic fertilization and control treatments reduced growth (Liu et al., 2013). Another possible reason of higher fungi population in manure treatment is the increased soil pH, as this was associated with increased fungal population in soil (Rousk et al., 2010). Increased fungi/bacteria ratio have been linked to increases in soil C and ecological buffering capacity (Bossio and Scow, 1998) as well as various organic amendments, such as DM (Wei et al., 2017), crop residue (Marschner et al., 2003), and green manure (Liu et al., 2009). Under P starvation arbuscular mycorrhizal fungi (AMF) forms symbiotic association with plant roots and increases the P availability to plants by the formation of mycorrhizal hyphae. In return it takes carbon from plants for its own growth and survival (Smith and Read, 2008). P deficiency in soil also significantly decrease AMF growth in tropical forests, because AMF initially were nutrient limited and application of phosphorus fertilizer led to increased AMF growth. Inorganic phosphorus fertilizers application can

increase soil pH which is most often associated with increased AMF population (Liu et al., 2013).

Agricultural management practices such as DM application, crop rotations and cover crops can reportedly increase or maintain soil quality for long-term agricultural production, as organic amendment/crop residue may regulate bacterial communities (DeBruyn et al., 2011). Crop rotations, mono-cropping and cropping sequence diversity are also pivotal factors influencing bacterial community structure and species diversity (Sarrantonio and Gallandt, 2003). Continued demands for silage corn will likely result in increased continuous cropping acreage, as corn silage is palatable, has higher yields and energy content than many other forages (Staples, 2003), and offers relatively consistent quality, making it an attractive forage crop to livestock producers. However, limited knowledge exists on the short-term influence of silage corn cropping on soil microbial community composition under organic and inorganic P amendments. Consequently, the aim of this study was to determine the impact of organic and inorganic amendment on growth, forage yield, extracellular enzyme, soil microbiological community and quality of silage corn under cool climate conditions.

1.9. Dry matter yield and quality of silage corn under organic and inorganic P sources

Plant nutrition is one of the main factor influencing silage corn production and quality, and it plays an important role in ensuring the agronomic performance of forages (Moreno-Resendez et al., 2017). In fact, nutrient management is one of the key strategy for

increasing forage yield and quality in silage corn. N, P and K have great significance in nutrient management process because they are the macro-nutrient with the highest transcendence in yield as well as in the quality parameters of silage corn and green forages (Iqbal et al., 2014; Moreno-Resendez et al., 2017).

Dry matter yield of crops is a function of numerous interacting factors (G x E x M) which includes, crop genotypes (G), environment (E) (temperature, rainfall, radiation, transpiration etc.) and management (M) practices (fertilizers application, sowing dates, cultural practices, seeding methods, weed control etc.) (Martin et al., 2014). Therefore, selection of suitable genotypes/hybrids and appropriate nutrient sources are important factors to determine the forage yield and quality of silage corn in different climate conditions. Combined application of poultry litter and cattle manure with inorganic fertilizer produced similar DMY to inorganic fertilizers, perhaps due to residual and soil amelioration effects (Eghball et al., 2004; Hirzel et al., 2007b). Silage corn DMY potential is not only associated with nutrient sources, physiochemical and biological properties of soil but also uptake efficiency of crops. Furthermore, P based poultry litter and cattle manure application is a beneficial amendment that helps to recycle nutrients, improve soil quality and produced similar dry matter yield of silage corn to inorganic P fertilizer (Nazli et al., 2014). Nitrogen (N) based poultry litter and cattle manure application significantly reduced dry matter yield of silage corn and this may be attributed to N deficiency caused by the lower N availability of poultry litter and cattle manure and absence of supplemental inorganic nitrogen (Hirzel et al., 2007a; Nazli et al., 2014). Furthermore, authors suggested that fulfillment of N requirements through organic materials produced unfavorable effects

on soil fertility and the environment due to the accumulation of soil phosphorus and metal ions (Codling et al., 2008). Inorganic P fertilizer (Triple super phosphate) application had no effect on fresh and DMY of silage corn compared to control (no phosphorus application) (Ali et al., 2014), and this response might be due to fixation of applied P by calcium (Ca^{+2}), iron (Fe^{+2}) and aluminum (Al^{+3}) which reduced the availability of P to plants (Shen et al., 2011).

Leytem et al. (2011) conducted study under greenhouse conditions to determine the effects of three phosphorus sources mono-ammonium phosphate (MAP), compost and dairy manure) and four rates of P application (25, 50, 100 and 200 mg/kg) on nutrients uptakes in silage corn. Phosphorus uptake in silage corn increased with increasing P application rate, highest increase occurred with MAP application, while the manure and compost treatments showed non-significant difference. MAP application increased P uptake which could be attributed to enhanced P solubility and decrease in soil pH, both of which might have enhanced P uptake by plants (Mengel and Kirkby, 1987). Manure and compost contains iron which react with P and forms Fe phosphates thereby reducing P solubility, and Ca precipitated with orthophosphate to form insoluble Ca-P precipitates. Under low P soils potassium (K) uptake increased for all treatments (MAP, manure and compost), whereas compost exhibited the highest K uptake in silage corn. Potassium uptake increased with increasing P application rate for all treatments under high P soils (Leytem et al., 2011).

On low P soil, calcium uptake increased under all treatments, while the highest Ca uptake was noticed in MAP, and it decreased significantly on high P soils with increasing rate of manure and compost. Reduction of Ca uptake in silage corn under manure and compost treatment could be related to cation competition with K and by the formation of Ca- P precipitates (Leytem et al., 2011). Magnesium uptake increased with increasing application rates of all treatments and highest uptake occurring in the MAP treatment. Under high P soil magnesium (Mg) uptake increased with increasing application rate of MAP and manure up to 100 mg/kg, above which leveled off for MAP and reduced for manure (Leytem et al., 2011). High K uptake may inhibit Mg uptake due to cation competition and balance between K, Ca and Mg uptake is a concern from an animal health prospective as forages with K:(Ca+Mg) ratios greater than 2.2:1 cause grass tetany in ruminant (Grunes et al., 1970). They found that ratio exceeds 2.2:1 for all treatments and rates, but it is important to keep in mind that silage corn harvested only after 3 weeks and therefore this may not represent the ratio in silage corn at maturity (Leytem et al., 2011)

Acid detergent fibers (ADF) represent the energetic values of silage corn (Moreno-Resendez et al., 2017),because it is constituted from cellulose, lignin and proteins, and is the component that is most related to forage digestibility (Castillo-Jimenez et al., 2009), the more ADF contents, the less digestibility of the forages (Castillo-Jimenez et al., 2009; Moreno-Resendez et al., 2017), therefore, good quality forage crops must contains < 28% ADF contents (Gallegos-Ponce et al., 2012). Organic fertilizer source produced 35.9% ADF in silage corn compared to inorganic fertilization which produced 27.2% ADF (Moreno-Resendez et al., 2017). Neutral detergent fibers (NDF) in forage crops negatively

correlate with intake and digestibility (Oramas-Wenholz and Quila, 2007), so to obtain high energy silage it is necessary to ensure <50% NDF contents in silage corn (Gallegos-Ponce et al., 2012). Organic fertilization produced 53.5% NDF in silage corn, whereas inorganic fertilizer produced 42.6% NDF (Moreno-Resendez et al., 2017). Nitrogen based organic fertilizer application (poultry liter and cattle manure) produced the highest ADF, NDF and lower crude protein (CP) compared to inorganic nitrogen treatment (Nazli et al., 2014). This might be attributed to differences in N content among poultry litter and cattle manure treatments because that strongly influence fiber content as well (Johnson et al., 2001). Combined application of inorganic P with poultry litter and cattle manure produced similar forage quality (ADF, NDF and CP) compared to inorganic P fertilization. Fiber contents significantly reduced with increasing rate of nitrogen fertilizers or nitrogen content in the plant tissue of silage corn (Nazli et al., 2014).

Carbohydrates compounds includes (free sugars, fructans, hemicellulose and cellulose) and classified as fibrous (structural) and non-fibrous (non-structural) carbohydrates (Martinez-Marin, 2008; Moreno-Resendez et al., 2017). Non-fibrous carbohydrates (NFC) were significantly increased by inorganic fertilization compared to organic fertilization. Reduction in silage corn digestibility was correlated with high values of NDF, not the NFC contents that was high in organic fertilizer treatments. (Moreno-Resendez et al., 2017). NFC contents of silage corn were 32.5% lower with organic fertilizer application compared to inorganic fertilizer application (Moreno-Resendez et al., 2017). Similarly, organic fertilization produced higher total digestible nutrients (TDN) (47%) compared to 46% with inorganic fertilization. It has been observed from the

literature reported above that organic and inorganic P sources influenced the quality indices of silage corn cultivated in different environments.

1.10. Objectives

Therefore, present study was conducted with following objectives;

i. To determine the agronomic performance (leaf area, chlorophyll contents, photosynthesis rate and forage yield) of silage corn amended with organic and inorganic P sources in podzolic soils under cool climate conditions
ii. To elucidate the effects of organic and inorganic P sources on soil available P, enzyme activities, and microbial communities.
iii. To assess the relationship between agronomic performance, biochemical attributes and active microbial communities.
iv. To investigate the effects of organic and inorganic P sources on forage quality of silage corn in cool climate.

1.11. References

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1.12. Co-authorship statement

Manuscripts based on the chapter 2, entitled “Effects of organic and inorganic phosphorus amendments on the agronomic performance, biochemical and microbial characteristics of silage corn in podzolic soil” and chapter 3 “Evaluating the forage production and quality of silage corn under organic and inorganic phosphorus sources” will be submitted to PLOS One and European Journal of Agronomy (Ali, W., Ashiq, W., Nadeem, M., Zaeem, S. Gillani, M. S. S., Thomas, R., Unc, A., Kavanagh, V., Cheema, M. 2018). Waqas Ali, the thesis author will be the primary author and Dr. Cheema (supervisor), will be the corresponding and the last author. Dr. Adrian Unc (co-supervisor) and Dr. Raymond Thomas (committee member) will be sixth and seventh authors, respectively. Dr. Kavanagh, research collaborator, Department of Fisheries and Land Resources will be the eighth author. For the work in Chapter 2 and chapter 3, Dr. Cheema wrote the research grants, developed the layout of this research field trial and helped in results and discussion interpretation. Mr. Waqas was responsible for the data collection, analysis, and writing of the manuscript. Dr. Nadeem, Mr. Waqar, Mr. Zaeem, assisted in experimental set up and seeding the field experiment and sample preparation. Dr. Thomas provided specific guidance in lab analysis and Dr. Kavanagh helped in experimental layout and seeding of the experiment. Dr. Cheema supervised overall experimental activities, edited and reviewed manuscript.

Chapter 2

2. Effects of organic and inorganic phosphorus amendments on the agronomic performance, biochemical and microbial characteristics of silage corn in podzolic soil

2.1. Abstract

Phosphorus (P) is the 2nd most important mineral and limiting nutrients for plant growth, development and productivity. Inorganic P fertilizer application to soil is predominantly bound to Al^{+3} and Fe^{+2} in acidic soils or with Ca^{+2} in alkaline soils, thereby reducing its availability to agricultural crops. Plants can improve phosphorus use efficiency (PUE) by modulating their root architecture including root morphology, topology and distribution patterns. Plant roots exude organic acids, acid phosphatase and some specific signaling substances, which are key drivers of P mobilization, solublization and acquisition in plants. Dairy manure application to agricultural soils can improve physiochemical properties of soil, and nutrients cycling by enhancing enzymes activities and soil microbial community structures leading to improved P availability in crops. This study aims to investigate the effects of organic and inorganic P sources on agronomic performance of silage corn genotypes, soil biochemical attributes and active microbial community composition in podzolic soils under cool climatic conditions. Experimental treatments were four P sources: [P_0 (control); P_1: dairy manure with high P conc.; P_2: dairy manure with low P conc.; P_3: inorganic P and five silage corn genotypes (Fusion RR, Yukon R, A4177G3RIB, DKC 23-17RIB, DKC 26-28RIB) and laid out in randomized complete block design. Agronomic performance (leaf area, chlorophyll contents, photosynthesis, shoot dry weight, root shoot

ratio and dry matter yield), rhizosphere soil biochemical attributes (pH, phosphatase activity and soil available P), and microbial community composition were measured. Yukon R showed superior agronomic performance and produced 28% and 60% higher dry matter yield than A4177G3RIB in 2016 and DKC23-17RIB in 2017, respectively. High P manure application increased the dry matter yield by 28% and 33%, acid phosphatase activity (Ap-ase) by 29% and 44%, soil available P by 60% and 39% compared to control in 2016 and 2017 respectively. High P manure application significantly increased Gram negative (G-) bacteria, fungi, eukaryotes, ΣB-PLFAs and ΣPLFAs, compared to inorganic P and control. Yukon R and DKC 26-28RIB had higher active fungal biomass, bacteria as well total in their root rhizospheres regardless of P sources compared to the other genotypes evaluated. Pearson correlation analysis demonstrated a positive relationship between active microbial community structure and the agronomic performance of the silage corn.

2.2. Introduction

Fertilizer application is an important practice that affects soil quality, health, fertility and sustainability of agricultural production systems. Low inputs of organic materials and excessive use of mineral fertilizers generally reduced soil organic carbon (SOC) contents, with a consequent decline in soil quality, health, fertility and crop productivity (Zhang et al., 2015). To maintain and improve soil quality, management practices such as manure application, no tillage, crop rotation and crop residue incorporation have been very productive in different cropping systems (Huang et al., 2015; Wang et al., 2015; Yan et al., 2013). Inorganic fertilization is not an option to improve SOC (Cai et al., 2014). Therefore,

enhancing the sustainability of cropping systems involves the integrated use of organic and inorganic inputs to promote the soil ecosystem function and interactions of the soil microbial community in providing nutrients for plant growth (Drinkwater and Snapp, 2007). The appropriate utilization of organic and inorganic amendment, such as DM and inorganic fertilizer application for instance, could be a useful approach in restoring SOC, soil fertility and sustainability (Cai et al., 2014; Sharpe et al., 2004), and therefore can increase plant nutrients availability and enhance soil microbial biomass, activity and diversity (Mandal et al., 2007; Wang et al., 2008).

Organic fertilizer amendment and management can substantially improve soil structure (Papadopoulos et al., 2014), help retain C in the surface soil, and increase crop yields in different cropping systems (Bhattacharyya et al., 2015). Applications of organic materials (e.g. green manure, dairy or livestock manure, and crop residue/straw) can reduce the required amounts of chemical fertilizers and compensate for soil C losses caused by land-use changes (Almagro and Martínez-Mena, 2014). Thus, amending soil with organic materials is a promising strategy to build-up C levels, improve soil quality, health and active microbial community structure in low fertility and shallow soils (Wang et al., 2015; Yuan et al., 2014). Dairy manure is historically known as a rich source of nitrogen (N), phosphorus (P), and potassium (K) and micronutrients (Hirzel and Walter, 2008). Dairy manure application increases soil microbial biomass being a rich source of organic matter and energy for microbes (Mikha and Rice, 2004). Soil microbes includes bacteria, archaeal, fungi and protozoa all known to produce phosphatases (George et al., 2002), which may increase the solubilization of organic P compounds and making them available to the

plants. Furthermore, DM application improve plant physiological characteristics and dry matter yield by improving phosphorus use efficiency (PUE) and nitrogen use efficiency (NUE) (Bernier et al., 2014). The biochemical parameters include variables that are directly related to microbial activity (microbial biomass carbon, soil respiration etc.) (Dinesh et al., 2010), and extracellular enzymes involved in the carbon (C), nitrogen (N) and phosphorus (P) cycles in soil, play a major role in degradation of organic matter, mineralization and availability of plant nutrients (Gil-Sotres et al., 2005). Most organic manures added into soil contain monomeric and polymeric compounds (such as sugars, starches, amino acids, urea, ammonium salts, fats, oils, waxes, proteins, humus, hemicellulose, cellulose, lignin, minerals), and thus the decomposition of these organic matter depends on the microbial production of extracellular enzymes and their break down should occur before taking up of low molecular weight organic molecules by microbial cells (Allison and Jastrow, 2006; Nannipieri et al., 2012; Rashid et al., 2013).

A variety of microbial activities can be considered as soil quality indicators such as the active microbial biomass and diversity in soil under normal conditions and in response to perturbations or stressors (Anderson, 2003; Bending et al., 2004; Kennedy and Smith, 1995). Soil microbial communities are responsible for cycling nutrients, regulating gas exchange, inducing micro aggregation, and altering the biochemical soil environment (Mikha and Rice, 2004; White and Rice, 2009). All these microbial functions drive sustainable soil health and ultimately crop productivity. Consequently, soil biological diversity and richness is seen as a key tenet of soil health, particularly determinations of bacterial assemblages may be one such metric for assessing soil quality and improving soil

fertility in agricultural production systems (Ashworth et al., 2017). Most studies focused on individual effects rather than combining effects of specific organic amendments on soil physicochemical properties. Biological properties are the most sensitive indicators of changes in the soil quality in different cropping systems due to their rapid responses to environmental changes (Lima et al., 2013). Consequently, these biological properties might be particularly useful for characterizing soil fertility and quality changes in short-term experiments. Information about the agronomic performance, biochemical and microbial communities' responses to organic and inorganic P fertilizer in podzolic soil under cool climatic region remains limited and needs to be investigated.

Phospholipid fatty acids (PLFAs) obtained from the membrane of microorganisms living in the soil are useful biomarkers that can be used to assess or provide estimates of the active soil microbial community composition, biomass and diversity under normal conditions and in response to land use changes, different types of management systems or stressors (Stark et al., 2007; Helgason et al., 2010). The microbial community, size and activity was observed to vary with different fertilizer managements, and the effects on soil fertility and productivity have been demonstrated using soil microbial PLFAs (Ai et al., 2012). Increases in the fungi/bacteria ratio have been linked to increases in soil C and ecological buffering capacity (Bossio and Scow, 1998; De Vries et al., 2012) in response to organic management (Bossio and Scow, 1998) as well as various organic amendments, such as livestock manure (Ling et al., 2014), crop residue (Marschner et al., 2003) and green manure (Liu et al., 2009). Agricultural management practices such as DM application, crop rotations and cover crops can reportedly increase or maintain soil quality

for long-term agricultural production, as organic amendment/crop residue may regulate bacterial communities (DeBruyn et al., 2011). The interacting factors of residue quality, quantity, and recalcitrance level associated with crop rotations can induce shifts in composition and frequency of archaeal and bacterial composition (White and Rice, 2009). Crop rotations, mono-cropping and cropping sequence diversity are also pivotal factors influencing bacterial assemblages and species diversity. High protein-containing biomass from soybeans and legume crops reportedly produce more labile residues than high C:N cereals such as corn (Sarrantonio and Gallandt, 2003). Continued demands for silage corn will likely result in increased continuous cropping acreage, as corn silage is palatable, has higher yields and energy content than many other forages (Staples, 2003). It also offers relatively consistent quality, making it an attractive forage crop to livestock and dairy industry. However, little information exists on the short-term influence of continuous silage corn cropping, on soil biochemical and microbial community composition under organic and inorganic P amendments. Consequently, the aim of this study was to determine the effects of organic and inorganic P fertilizer application on agronomic performance, extracellular enzyme activities and microbial composition and abundance in podzolic soils under northern agriculture production systems. We hypothesized that organic and inorganic P fertilizer amendments will improve enhance extracellular enzyme activities and create phylogenetically diverse active microbial communities in soil, thereby increasing phosphorus availability and improve agronomic performance of silage corn.

Podzolic soils typically have a coarse-sandy texture and high acidity, with pH in the topsoil layer around 4 to 4.5 (Sauer et al., 2007). These conditions signal a poor nutrient

supply for agricultural crops. Moreover, prior studies have shown that nutrient availability in podzols may not conform to classical tenets of soil chemistry (Grand and Lavkulich, 2013). As agricultural development is on fast track in the province, so there is a need to enhance our knowledge and understanding on nutrient availability, enzyme activities and soil microbiological composition and abundance in podzolic soils under organic and inorganic amendments, so that nutrient management practices may be adapted to allow an eco-environment friendly and economically sustainable agricultural production system. This study will investigate the changes in soil fertility under different organic and inorganic P amendments by measuring soil biochemical and microbiological properties and thus provide strong evidence for improving the soil quality and health and agronomic performance of silage corn in podzolic soil. Our objectives were to evaluate the effects of organic and inorganic P sources and genotypic response on (i) agronomic performance (leaf area, leaf chlorophyll contents, net photosynthesis and dry matter yield) of silage corn (ii) soil available P, enzyme activities, and microbial communities' abundance (iii) relationship between agronomic performance, biochemical and active microbial communities.

2.3. Material and Methods

2.3.1. Experimental site and treatments

A field research trial was conducted at Pynn's Brook Research Station, Pasadena (49.087° N, 57.541° W), during 2016 and 2017 growing seasons. Before conducting the experiment, composite soil samples were collected from the experimental site and were

sent to the Soil, Plant and Feed Laboratory, Department of Fisheries, and Land Resources, St. John's NL for complete nutrients analysis. Soil analyses report can be seen in Table 2.1. Soil texture was determined to be loamy sand with 82.0% sand, 11.6% silt, and clay particles were 6.4%. Weather data (maximum temperature, minimum temperature, average temperature, and precipitation) was obtained from a weather station located adjacent to the experimental site at Pynn's Brook Research Station. Mean average temperature during 2016 and 2017 growing season was 12.20 ^{o}C and 11.81 ^{o}C respectively. However, 2017 growing season received only 496 mm rain and was much dryer compared to 2016, where 704 mm rain was recorded (Figure 2.3.1, Table 2.2). The experimental treatments were four P sources and five silage corn genotypes and was laid out in randomized complete block design (RCBD) with three replications. Each experimental treatment plot was 4.8 × 1.5 meters with row to row distance 0.76 m orientated in east-west directions. Silage corn genotypes were chosen on the basis of corn heating units (CHU) requirement as western Newfoundland falls in northern climate production systems, and needs low heating unit crops. Details about silage corn genotypic CHU, genetic traits and suppliers are given in Table 2.3. Two phosphorus manure sources used in this experiment were selected on the basis of P concentration in manure obtained from two dairy farms. Details about the P sources along with rate of application are given in Table 2.4. For determining the high and low P concentration in DM, well-agitated DM samples were collected from all ten dairy farms across western NL before the start of growing seasons to analyze their P concentrations. DM samples were sent to soil, plant and feed laboratory, Department of Fisheries, and Land Resources, St. John's NL. Thereafter, two DM samples were selected

based on the high and low P concentration and were designated as P_1 (high P) and P_2 (low P), and were sourced from Larch Grove Farm and Rideout's Dairy Farm respectively (Table 2.5). Details about P sources is given in Table 2.4, whereas, complete manure analysis report of both dairy farms is presented in Table 2.5.

Table 2.1 Soil analysis report of experimental site in 2016 and 2017

Soil Properties	2016	2017
Soil pH	6.4	6.8
Phosphorus (mg/L)	81	74
Potassium (mg/L)	38	49
Calcium (mg/L)	1256	1120
Magnesium (mg/L)	265	218
Organic matter (%)	2.98	3.01
Sulphur (mg/L)	14	15
Zinc (mg/L)	0.6	1.3
Copper (mg/L)	1.1	2.1
Sodium (mg/L)	7	6
Iron (mg/L)	150	130
Boron (mg/L)	0.1	0.2
Manganese (mg/L)	18	16
Aluminum (mg/L)	1507	1409

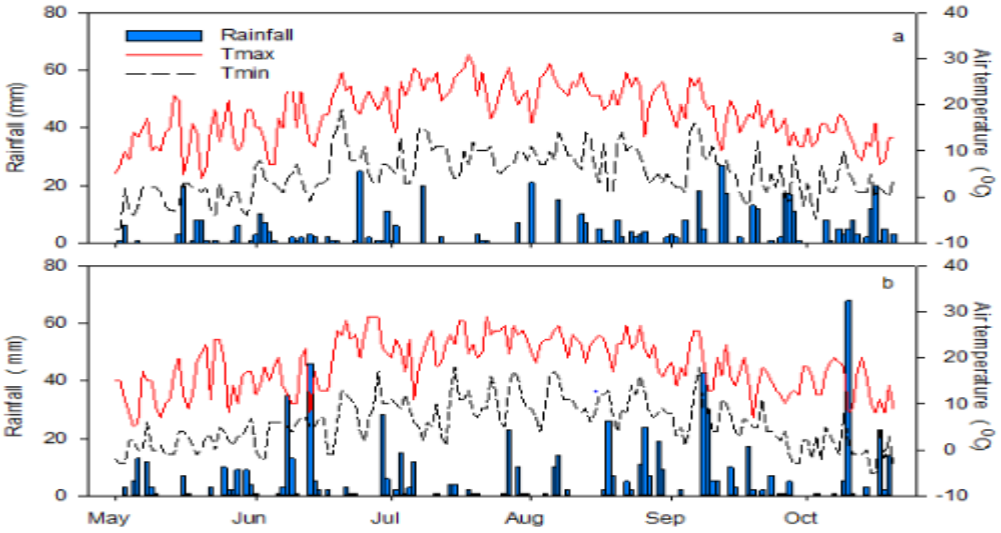

Figure 2.3.1: Weather conditions at Pynn's Brook Research Station, Pasadena during 2017 (a) and 2016 (b) growing season

Table 2.2 Weather conditions at Pynn's Brook Research Station, Pasadena during 2016 and 2017 growing seasons

Growth period	Mean maximum temperature (°C)		Mean minimum temperature		Mean average temperature		Rainfall (mm)	
	2016	2017	2016	2017	2016	2017	2016	2017
May 1-15	12.06	12.8	0.26	-1.46	6.16	5.66	36	11
May 16-31	16.06	1306	2.86	0	9.37	6.53	47	47
June 1-15	14.86	15.33	4.4	3.53	9.63	9.43	107	35
June 16-30	23.26	21.46	7.73	8.4	15.5	14.93	41	45
July 1-15	21	23.33	7.93	8.8	14.46	16.06	42	29
July 16-31	24.81	24.18	10.93	8.31	17.87	16.25	40	12
August 1-15	23.06	23.8	10.06	9.46	16.56	16.63	27	53
August 16-31	21.75	21.75	10	6.87	15.87	14.5	112	32
September 1-15	18.4	18.4	7.46	7.53	12.93	13.3	98	80
September 16-30	14.2	14.2	3.26	2.86	8.73	9.1	36	76
October 1-15	14.05	14.05	0.4	2.4	7.22	7.6	118	76
							704	**496**

Table 2.3 Basic information about silage corn genotypes used in the experiment

Corn silage genotype	Corn heat units	Genetic trait	Supplier
Fusion RR	2200	RR2	Brett Young, Canada
Yukon R	2150	RR2	Brett Young, Canada
A4177G3RIB	2175	VT3P/RR	Pride Seeds, Canada
DKC23-17RIB	2075	VT2P	DEKALB, Canada
DKC26-28RIB	2150	VT2P	DEKALB, Canada

RIB = Refuge is in the bag; RR = Roundup Ready; VT2P = VT Double PRO® RIB Complete; VT3P= VT TriplePRO® RIB Complete insect protection; RR2 = Resistance gene to Roundup® and Factor 540®

Table 2.4 Details about P sources used in the experiment

P sources	
P_0	Control
P_1	Manure with high P (0.6 kg P_2O_5 1000 L^{-1}) @ 30000 L ha^{-1}
P_2	Manure with low P (0.3 kg P_2O_5 1000 L^{-1}) @ 30000 L ha^{-1}
P_3	Inorganic P (Triple super phosphate) @ 110 kg ha^{-1}

Table 2.5 Composition of diary manure sources used in the experiment

	Larch Grove Dairy Farm (High P Manure)		Rideouts Dairy Farm (Low P manure)	
Manure Properties	2016	2017	2016	2017
Dry matter (%)	9.33	10.9	3.57	1.7
pH	6.8	6.8	7	7.1
Nitrogen (kg/1000 L)	1.5	1.9	0.5	0.5
Phosphorus (kg/1000 L)	0.6	1.7	0.3	0.3
Potassium(kg/1000 L)	4.1	4.0	1.3	1.3
Total Calcium (%)	0.164	0.19	0.059	0.042
Total Magnesium (%)	0.069	0.077	0.026	0.018
Total Iron (ppm)	49	68	19	7
Total Manganese (ppm)	23	21	9	5
Total Copper (ppm)	4.7	4.5	33	20
Total Zinc (ppm)	17	21	8	5
Total Boron (ppm)	3	3.4	1	0.5
Total Sodium (ppm)	911	904	275	241

2.3.2. Crop husbandry

Silage corn genotypes were seeded on May 25th and May 23rd during 2016 and 2017, using SAMCO systems (SAMCO 2200 Agricultural Manufacturing, Limerick, Ireland). SAMCO 2200 system has the ability to seed two corn rows and simultaneously cover the seeded plots with plastic sheet. The partially perforated plastic sheet provides cover over about 1 m width for two adjacent corn rows to enhance the CHU during early crop growth for better germination and seedling establishment. One week prior to seeding, land was disked and crop was seeded @ 90900 plants ha^{-1}. In each growing season, fresh manure samples were sourced out from Larch Grove and Rideout's Dairy Farms and applied to the respective DM treatment plots @ 30,000 L ha^{-1} according to the local dairy farmer's practices. The applied DM was incorporated thoroughly in the top 15 cm soil layer a day before seeding. It is pertinent to mention that experimental plots were kept at the same locations during both years to avoid any treatment effects. The remaining crop

nutrient requirements were fulfilled through inorganic fertilizers keeping in view the soil nutrient status, DM analyses reports and regional recommendations for silage corn. Roundup WeatherMax® (Monsanto Canada Inc.) was applied according to the instructions given on label during both years to control the weeds, and crop was harvested at black layer stage on October 18 and 13 during 2016 and 2017, respectively. All agronomic performance parameters, plant and soil samples for biochemical and microbial community analysis were collected using standard procedures.

2.3.3. Agronomic performance parameters

Leaf area, chlorophyll contents, photosynthesis rate, root-shoot dry weight and root-shoot ratio were measured at six leaf, twelve leaf, tasseling and black layer stages of the silage corn. However, plant height and DMY were recorded at harvesting stage of the crop. A portable leaf area meter LI-3000 C system (LICOR Biosciences), was used to measure the leaf area on the basis of length and width of area. The final data set was downloaded using LI-3000C software (www.licor.com). Chlorophyll contents of fully expanded leaves from top were measured, using SPAD 502 Plus chlorophyll meter (Konica Minolta Europe) and measurements were taken between 8:30-14:00 h. Leaf photosynthetic rate was measured from the fully expanded leaves from the top between 8:30 and 14:00 h using LI-6400XT portable Photosynthesis system (LI-COR Biosciences, Lincoln, Nebraska). Photosynthetic system used blue light (475 nm) and red light emitting diodes (LED) (630 nm) as a light source mounted on a two square centimeter. The measurements were recorded when a steady state at approximately 2-4 minutes were obtained at 2000

μmol photon m^{-2} s^{-1} PAR and leaf cuvette temperature was set to 25 ºC. The CO_2 entering in the cuvette was maintained at 400 μmol mol^{-1} while the relative humidity varied between 40–50%. The photosynthesis data was downloaded using LI-6400XT software (LICOR – 6400XT instruction Manual, version 5, LI-COR Inc., Lincoln, Nebraska, USA). Three plants from each treatment were uprooted to measure root-shoot dry weight. After uprooting, the root and shoot were separated from each plant sample then stored in polythene bag and immediately transported to the laboratory, and adhered soil from the roots was removed gently using brush followed by washing with tap water. After washing shoots were chopped in to small pieces and dried in forced air oven (Shell Labs USA) at 65 ºC till a constant weight was obtained. The oven dry weight was recorded for estimating dry weight per plant. Root shoot ratio was calculated by using the following formula:

Root shoot ratio = root dry weight (g) / shoot dry weight (g)

Plant height was measured using a meter rod. Briefly, six plants from each treatment plot were chosen randomly for measuring plant height, and the mean values were calculated for each replicate. For measuring the DMY, crop was harvested manually from one square meter area of each treatment plot at black layer stage. The fresh biomass was recorded with weighing balance, and then samples were chopped into small pieces to dry in a forced air oven (Shell Labs USA) at 65 °C until constant dry weight was attained. The dry matter yield % was determined as follows:

Dry matter = (weight of samples after drying / weight of samples before drying) x 100

Dry matter yield = Fresh biomass x % Dry matter

2.3.4. Soil sampling and analysis methods

Three plants from each treatment plot were uprooted, and the adherent soil was gently removed from all roots and mixed in to a composite sample. Soil samples were kept on ice in a cooler, immediately transported to the laboratory and sieved through 2 mm mesh to remove debris and other waste materials; then divided in to three subsamples. One sub-sample was air dried at 25 ^{o}C for determining soil available P and pH, one sub-sample was stored at 4^{o}C for biochemical analysis (enzymes) and the third sub-sample was stored at -20 ^{o}C for PLFAs to determine the active microbial community composition and abundance (the soil was freeze-dried before the determination of PLFAs).

2.3.5. Biochemical attributes

Soil pH was measured by extracting 10 g air dried soil with 20 mL of 0.01 M $CaCl_2$ (calcium chloride) in 50 mL polypropylene tubes. The soil solution was then mixed for 30 minutes on an orbital shaker (Innova™ 2300 Platform Shaker, New Brunswick Scientific, Canada) at 120 rpm, then allowed to stand for 1 hour and the pH was measured with a pH meter (METTLER TOLEDO, Canada) (Hendershot et al., 2006).

The acid phosphatase activity (Ap-ase) was based on the determination of p-nitrophenol phosphate (PNP) (Tabatabai and Bremner, 1969). Briefly, 1 g soil was extracted using 0.09 M 1 mL citrate buffer in 15 mL polypropylene tubes and centrifuged at 5000 rpm for ten minutes. A 50 µl aliquot of the supernatant was dispensed into a 96-

well microplate where 50 µl p-nitrophenol phosphate substrate and 30 µl citrate buffer was added to all the samples. The samples were then incubated at 37 ^{o}C for 30 minutes. After incubation, 20 µl of NaOH (sodium hydroxide) was dispensed in all wells to stop further reaction and the absorbance of the mixture measured at 450 nm with BioTek Synergy Cytation^{TM} 3 imaging reader. The absorbance was used to calculate the acid phosphatase activity in the sample and the values expressed in µmole p-nitrophenol g^{-1} soil min^{-30}.

Soil available P (SAP) was analyzed using the Mehlich 3 extraction method (Mehlich, 1984) where 2 g of soil was extracted with 20 mL of Mehlich 3 solution in 50 mL Erlenmeyer flask, The sample mixtures was shaken for 5 minutes on an orbital shaker at 120 rpm (Innova™ 2300 Platform Shaker, New Brunswick Scientific, Canada). After shaking the solution was filtered using Whatman 42 filter papers (Sigma Aldrich, ON. Canada). Aliquots of the filtrate was diluted 50 times and then analyzed with AA3 Continuous Flow Analytical System (AA3HR, SEAL Analytical, USA) to determined soil available P, expressed in mg/kg as follow:

Mehlich 3 extractable P (mg/kg) = [Concentration of P in Mehlich 3 extract, mg/L] x [0.020 L extract / 0.002 kg soil]

2.3.6. Phospholipids fatty acids analysis

Phospholipids fatty acids (PLFAs) were determined according to the methods reported by Folch et al. (1957) and Gomez-brandon and Dominguez (2010). Total fatty acids were extracted from 4 g soil with 10 mL chloroform-methanol, 2:1 (v/v) in 20 ml

glass vials. The samples were homogenized with a sonicator for 5 minutes (amplitude 50; pulse on time: 5 seconds; and pulse off time; 10 seconds), and mixture was allowed to separate at room temperature for 24 hours. The supernatant was filtered and collected in glass vials, and then evaporated to dryness under a stream of oxygen free N_2 gas. The total lipid extracts obtained were dissolved in 2 mL chloroform and fractionated into neutral lipids, glycolipids and phospholipids, with chloroform (2.5 mL), acetone (4 mL) and methanol (2.5 mL), by means of solid phase extraction (SPE) on silicic acid columns (Discovery ^{R} DSC- Si SPE tube, 50 µm, 70 Å, 100 mg/1mL). The separated phospholipids were evaporated to dryness under a stream of oxygen free N_2 gas. Phospholipids extracts were dissolved in 500 µl of methyl tertiary-butyl ether. Aliquots (100 µl aliquots) were taken from the 500 µl extracts and placed in a screw-cap vials with 50 µl of derivatization agent trimethyl sulfonium hydroxide (TMSH), vortex mixed for 30 s and allowed to react for 30 minutes. 10 µl of internal standard methyl nonadeconate (19:0 @ 160 ug/ml) were added to the extract of methylated PLFAs and the samples analyzed with Gas Chromatography-flame ionization detection (GC-FID).

2.3.7. Phospholipid fatty acids (PLFAs) analysis with GC-FID

PLFAs analysis was conducted on a Thermo Scientific Trace 1300 gas chromatography coupled to a flame ionization detector (Thermo Fisher Scientific, Waltham, MA, USA). The methylated fatty acids were separated with a DB-23 column (30 m × 0.32 mm × 0.25 µm; Agilent Technologies, Canada) by supplying helium (He) as carrier gas at a continuous flow rate of 1 ml/min. The GC injector was run in splitless

mode and 1 µl of each samples was applied to the injection system using a Tri-plus auto-sampler. The initial oven temperature was 50 ^{o}C (1 min hold), then increased using a heating rate of 20 °C/min to 175 °C, kept at 175 °C for 1 min, and further increased at a rate of 4 °C/min to 230 ^{o}C (hold up to 5 min). To identify the methylated PLFAs, the retention times and mass spectra were compared with those obtained from commercial standards (NIST database) (Thermo Scientific, ON. Canada, Supelco 37 Component Fatty acid methyl ester (FAME) mix, and Bacterial acid methyl ester (BAME) Mix purchased from, Sigma Aldrich, ON, Canada). Methylated PLFAs were quantified using internal standards and the results expressed in nmol g^{-1} soil. A total of 47 PLFAs were identified and 27 used to measure the total microbial biomass are mentioned in Table 2.6.

2.3.8. Statistical analysis

Data was analyzed using the statistical package Statistix 8.1 (Analytical software, Tallahassee FL 32317, USA) to determine the effects of phosphorus sources on agronomic performance of silage corn genotypes, soil pH, soil available P, acid phosphatase activity and soil microbial community structure. Least significance difference (LSD) method at the probability level of 0.05 was used to separate the mean differences of the treatments. Principal component analysis was done using a PAST (Paleontological Statistics 3.0) to determine the effects of genotypes and P sources on agronomic performance, soil biochemical parameters and the active soil microbial community, and its relationship among the variables. Sigma plot 14.0 (Systat Software Inc.) were used to make weather graph.

Table 2.6 PLFAs as microbial biomarkers

Taxonomic group	Biomarkers	References
Gram positive (G+)	C14_0	(Sheng et al., 2012)
	i-C15_0	(Wang et al., 2016; Zhang et al., 2016)
	a-C15_0	(Wang et al., 2016; Zhang et al., 2016)
	C15_0	(Huygens et al., 2011; Papatheodorou et al., 2012)
	i-C16_0	(Wang et al., 2016; Zhang et al., 2016)
	C16_0	(Kujur and Patel, 2014; Wu et al., 2015)
	C16_1n-7	(Brockett et al., 2012; (Wang et al., 2016)
	i-C17_0	(Wang et al., 2016; Zhang et al., 2016)
	C17_0	(Huygens et al., 2011; Papatheodorou et al., 2012)
	C18_0	(Brockett et al., 2012; Wu et al., 2015)
	C18_1n-9cis	(Bsrockett et al., 2012; Zhang et al., 2016)
Gram Negative (G-)	2OH_C10_0	(Lasater et al., 2017)
	2OH_C12_0	(Lasater et al., 2017)
	C16_0	(Kujur and Patel, 2014; Wu et al., 2015)
	C16_1n-7	(Brockett et al., 2012; (Wang et al., 2016)
	3OH_C12_0	(Kaur et al., 2005)
	cycloC17_0	(Wang et al., 2016; Zhang et al., 2016)
	C18_0	(Brockett et al., 2012; Wu et al., 2015)
	C18_1n-9_trans	(Moreno et al., 2017)
	C18_1n-9cis	(Brockett et al., 2012; Zhang et al., 2016)
	3OH_C14_0	(Papatheodorou et al., 2012)
	cycloC19_0	(Wang et al., 2016)
	C14_1n_5	(Zhang et al., 2016)
	C17_1n_7	(Gomez-brandon and Dominguez, 2010)
Fungi	C18_1n_9cis	(Brockett et al., 2012; Zhang et al., 2016)
	C18_2n_6cis	(Joergensen and Potthoff, 2005; Zhang et al., 2016)
	C18_3n_3	(Mckinley et al., 2005; Wu et al., 2015)
	C20_1n_9	(Li et al., 2016; Mckinley et al., 2005)
Protozoa	C20_0	(Schindlbacher et al., 2011)
	C20_3n_6	(Buyer and Sasser, 2012)
	C20_4n_6	(Wu et al., 2013)
Eukaryotes	C18_2n_6cis	(Joergensen and Potthoff, 2005; Zhang et al., 2016)
	C21_0	(Zelles, 1999)

2.4. Results

2.4.1. Agronomic performance parameters

2.4.1.1. Leaf area (cm^2 $plant^{-1}$)

Genotypes × phosphorus sources interaction had significantly ($p<0.05$) affected leaf area (LA) during 2016 (Table 2.7). Highest LA was recorded in Yukon R (3408 cm^2 $plant^{-1}$) when high P concentration manure was applied compared to lowest LA (2046 cm^2 $plant^{-1}$) was produced by A4177G3 RIB in control treatment (Table 2.9). Interaction between genotypes and P sources (G x P) on LA was non-significant in 2017 (Table 2.8), however, genotypes and P sources had significantly ($p<0.01$) influenced LA (Table 2.8). Individual comparison of treatments means showed that Yukon R produced significantly higher LA (3244 cm^2) while the lowest LA (2040 cm^2) was noticed in DKC 23-17RIB. Phosphorus sources also showed significant effects on LA, and highest LA (2884 cm^2) was noted in high P concentration manure treatment. Lowest LA (2120 cm^2) was recorded in P_0 treatment (Table 2.9). High P manure increased 36% LA compared to control treatment.

2.4.1.2. Chlorophyll contents (SPAD values)

Chlorophyll contents were significantly ($p<0.05$) influenced with Genotypes × phosphorus sources interaction during 2016 (Table 2.7). Highest chlorophyll contents (48.66) were noted in Yukon R under P_1 treatment, and the lowest were recorded in A4177G3RIB under control treatment (Table 2.10). In 2017, Genotypes × phosphorus

sources had significant ($p<0.01$) effects on chlorophyll contents. Pattern of highest and lowest chlorophyll contents were the same in 2016.

2.4.1.3. Photosynthesis rate (µmol CO_2 m^{-2} s^{-1} $plant^{-1}$)

Genotypes × phosphorus sources had significantly ($p<0.05$) affected net photosynthesis during 2016 (Table 2.7). Higher photosynthesis rate (34.43 µmol CO_2 m^{-2} s^{-1} $plant^{-1}$) was recorded in Yukon R when fertilized with high P manure compared to lowest photosynthesis (25. 67 µmol CO_2 m^{-2} s^{-1} $plant^{-1}$) was observed in A4177G3 in control treatment (Table 2.11). In 2017, genotypes × phosphorus sources showed non-significant effects on photosynthesis rate. However, genotypes and P sources had significantly ($p<0.001$) influenced photosynthesis rate (Table 2.8). Individual comparison of treatment means showed that higher photosynthesis (29.28 µmol CO_2 m^{-2} s^{-1} $plant^{-1}$) was noted in Yukon R, which was statistically at par with DKC26-28RIB and Fusion RR, compared to the lowest (25.20 µmol CO_2 m^{-2} s^{-1} $plant^{-1}$) exhibited by DKC23-17RIB (Table 2.11). High P manure showed greater photosynthesis (28.99 µmol CO_2 m^{-2} s^{-1} $plant^{-1}$) compared to control (26.78 µmol CO_2 m^{-2} s^{-1} $plant^{-1}$). Surprisingly, inorganic P source and control treatment produced similar photosynthesis.

2.4.1.4. Shoot dry weight (g $plant^{-1}$)

Statistical analyses demonstrated that Genotypes × Phosphorus sources had significantly ($p<0.01$) affected shoot dry weight during 2016 (Table 2.7). Yukon R produced higher shoot dry weight (169.17 g $plant^{-1}$) when amended with high P manure

and A4177G3RIB genotype exhibited low shoot dry weight (98.67 g plant^{-1}) in control treatment. Genotypes × Phosphorus sources interaction had non-significant effects on shoot dry weight during 2017. Genotypes and P sources as individual factor significantly ($p<0.001$) influenced shoot dry weight (Table 2.8). Individual comparison of treatment means for genotypes showed that Yukon R produced highest shoot dry weight followed by Fusion RR and lowest was exhibited by DKC23-17 RIB. However, Fusion RR, DKC 26-28RIB and A4177G3 RIB were statistically at par with each other. Among P sources, high P manure produced higher shoot dry weight followed by low P manure compared to control treatment which produced lowest shoot dry weight. Shoot dry weight of silage corn was 35% higher with high P manure compared to control.

2.4.1.5. Root shoot ratio (plant^{-1})

Root: shoot ratio was significantly influenced by Genotypes × Phosphorus sources interaction in 2016 & 2017 (Table 2.7 & Table 2.8). In both years highest root shoot ratio was recorded in control treatment. However, in 2016, highest root shoot ratio (0.96 plant^{-1}) was produced by A4177G3RIB followed by DKC 26-28RIB, and lowest was also produced by DKC26-28RIB with inorganic P source. In 2017, Fusion RR produced highest root-shoot ratio and lowest was observed in Yukon R with inorganic P source application (Table 2.13).

2.4.1.6. Plant height (cm)

Genotypes × Phosphorus sources interaction significantly ($p < 0.05$) affected plant height during 2016, however, G × P sources interaction was non-significant in 2017 (Table 2.7 & Table 2.8). Maximum plant height (236.33 cm) was attained by Yukon R in high P manure treatment, whereas minimum plant height (188.33 cm) was displayed by A4177G3RIB in control treatment (Table 2.14). During 2017, the interactive effect of G x P sources showed non-significant effects, however, genotypes and P sources as an individual factor had significant effects significant on plant height (Table 2.8). Individual comparison of genotype means showed that, Yukon R was the tallest and attained greatest plant height (194.87 cm) and A4177G3RIB produced lowest plant height (150.39 cm). Among P sources, high P manure treatment was the most effective treatment which produced tallest plants compared to shortest plant height was achieved in control treatment (Table 2.14).Both P manure treatments (high and low P manure) were statistically at par with each other.

2.4.1.7. Dry matter yield (Mg ha^{-1})

Genotypes, P sources and their interaction (G × P) had significantly ($p<0.01$) affected dry matter yield (DMY) during 2016 and non-significant interaction between G × P was noted in 2017 (Table 2.7 & Table 2.8). Manure with high P treatment led to produce high dry matter yield (21.94 Mg ha^{-1}) of Yukon R, and lowest DMY (13.12 Mg ha^{-1}) was recorded by A4177G3RIB in control treatment (Table 2.15). During 2017, among genotypes, Yukon R produced highest DMY of 14.99 Mg ha^{-1}, followed by DKC 26-28RIB

which produced 12.97 Mg ha^{-1} whereas, DKC23-17RIB produced lowest DMY of 9.34 Mg ha^{-1}. DKC 26-28RIB and Fusion RR were statistically at par in DMY production (12.97 and 12.66 Mg ha^{-1}). P sources showed highly significant ($p<0.001$) differences in DMY, and high P manure treatment produced higher DMY of 14.23 Mg ha^{-1} followed by low P manure application and lowest (10.67 Mg ha^{-1}) was recorded in control treatment. High P manure produced 33.39% higher DMY compared to control treatment (Table 2.15).

2.4.2. Biochemical attributes

ANOVA (Analysis of variance) showed that Genotypes × phosphorus sources significantly ($p<0.001$) affected soil rhizosphere pH during 2016 (Table 2.16). High pH was found in the rhizosphere of Fusion RR when inorganic P was applied, compared to lowest soil pH observed in DKC26-28RIB genotype in the same P treatment (Table 2.18). However, Genotypes × phosphorus sources on soil rhizosphere pH was significant in 2017 (Table 2.17). Comparison of treatment means showed that soil pH was highest in the rhizosphere of A4177G3 RIB genotype under inorganic P, and lowest was found in DKC23-17RIB under same treatment. Manure with high P centration significantly ($p<0.001$) increased soil rhizosphere pH and lowest was noted in control treatment (Table 2.18).

Table 2.7 ANOVA for agronomic performance parameters during 2016

Agronomic Parameters	Genotypes	Phosphorus sources	G x P	Coefficient of variation%
Leaf area	***	***	*	3.93
Chlorophyll contents	***	***	*	3.15
Photosynthesis rate	***	***	*	2.88
Shoot dry weight	***	***	**	4.52
Root shoot ratio	NS	***	**	8.14
Plant height	***	***	*	2.16
Dry matter yield	***	***	**	5.18

(*** Significant at $p < 0.001$, ** Significant at $p < 0.1$, *Significant at $p < 0.05$, NS= Non-Significant)

Table 2.8 ANOVA for agronomic performance parameters during 2017

Agronomic Parameters	Genotypes	Phosphorus sources	G x P	Coefficient of variation%
Leaf area	***	***	NS	13.28
Chlorophyll contents	***	***	***	5.73
Photosynthesis rate	***	***	NS	5.63
Shoot dry weight	***	***	NS	14.2
Root shoot ratio	***	**	*	19.54
Plant height	***	***	NS	5.55
Dry matter yield	***	***	NS	14.26

(*** Significant at $p < 0.001$, ** Significant at $p < 0.1$, *Significant at $p < 0.05$, NS= Non-Significant)

Table 2.9 Effects of phosphorus sources on leaf area (cm^2) of silage corn genotypes at tasseling stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
2016	P_0	2281.6 ^{j}	2600.3 ^{fgh}	2046.2 ^{k}	2548.3 ^{gh}	2454.6 ^{hij}	2386 ^{D}
	P_1	2700.8 ^{efg}	3408 ^{a}	2626.8 ^{fgh}	2991.2 ^{bc}	3164.6 ^{b}	2978 ^{A}
	P_2	2547.2 ^{gh}	3162.2 ^{b}	2487.5 ^{hi}	2725.1 ^{ef}	2968.4 ^{cd}	2778 ^{B}
	P_3	2349.7 ^{ij}	2810.8 ^{de}	2331.7 ^{ij}	2590.2 ^{fgh}	2833.6 ^{cde}	2583 ^{C}
	Means	2469.8 ^{D}	2995.3 ^{A}	2373.1 ^{E}	2713.7 ^{C}	2855.3 ^{B}	
2017	P_0	1897	3026.6	1881.8	1655	2141.4	2120 ^{C}
	P_1	2678.6	3683.8	2538.6	2578.7	2940.3	2884 ^{A}
	P_2	2522.5	3306.3	2333.5	2246.2	2508.3	2583 ^{B}
	P_3	2267.7	2962.5	1938	1682.6	2464.3	2263 ^{C}
	Means	2341.5 ^{BC}	3244.8 ^{A}	2173 ^{CD}	2040.6 ^{D}	2513.6 ^{B}	

(Common letter means no significant difference at $p < 5$ %)

Table 2.10 Effects of phosphorus sources on chlorophyll contents of silage corn genotypes at tasseling stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
2016	P_0	42 ^{efgh}	42.66 ^{def}	37 ^{j}	39.66 ^{i}	42.33 ^{defg}	40.73 ^{D}
	P_1	45.33 ^{b}	48.66 ^{a}	45.33 ^{b}	44.33 ^{bcd}	45.66 ^{b}	45.86 ^{A}
	P_2	45 ^{bc}	45.66 ^{b}	42.66 ^{def}	42.33 ^{defg}	44 ^{bcde}	43.93 ^{B}
	P_3	40 ^{hi}	45.33 ^{b}	40.33 ^{ghi}	41.33 ^{fghi}	43 ^{cdef}	42 ^{C}
	Means	43.08 B	45.58 A	41.33 C	41.91 C	43.75 B	
2017	P_0	41.84 ^{e}	44.22 ^{abcde}	42.56 ^{bcde}	34.3 ^{f}	42.34 ^{cde}	41.05 ^{B}
	P_1	44.04 ^{abcde}	46.98 ^{a}	45 ^{abcde}	45.96 ^{abcd}	46.22 ^{abc}	45.64 ^{A}
	P_2	42.08 ^{de}	46.6 ^{ab}	43.58 ^{abcde}	44.7 ^{abcde}	44.74 ^{abcde}	44.34 ^{A}
	P_3	41.62 ^{e}	46.64 ^{ab}	43.68 ^{abcde}	31.86 ^{f}	42.27 ^{cde}	41.22 ^{B}
	Means	42.4 ^{B}	46.11 ^{A}	43.71 ^{B}	39.20 ^{C}	43.89 ^{B}	

(Common letter means no significant difference at $p < 5$ %)

Table 2.11 Effects of phosphorus sources on photosynthesis rate (μmol CO_2 m^{-2} s^{-1} $plant^{-1}$) of silage corn genotypes at tasseling stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
2016	P_0	27.35 ^{k}	30.42 ^{efghi}	25.67 ^{l}	29.27 ^{hij}	30.62 ^{efgh}	28.67 ^{D}
	P_1	31.65 ^{cde}	34.43 ^{a}	31.61 ^{cde}	32.12 ^{bcd}	33.5 ^{ab}	32.66 ^{A}
	P_2	29.91 ^{fghij}	33.02 ^{abc}	29.11 ^{ij}	30.32 ^{efghi}	31.23 ^{def}	30.72 ^{B}
	P_3	29.57 ^{ghij}	32.39 ^{bcd}	27.47 ^{k}	28.49 ^{jk}	31 ^{defg}	29.78 ^{C}
	Means	29.62 C	32.56 A	28.46 ^{D}	30.05 ^{C}	31.58 ^{B}	
2017	P_0	27.17	30.34	25.45	22.834	28.087	26.78 ^{B}
	P_1	29.95	29.13	28	27.503	30.36	28.99 ^{A}
	P_2	29.34	29.22	27.67	25.835	30.074	28.43 ^{A}
	P_3	28.06	28.43	26.38	24.637	27.531	27.01 ^{B}
	Means	28.63 ^{A}	29.28 ^{A}	26.88 ^{B}	25.20 ^{C}	29.01 ^{A}	

(Common letter means no significant difference at $p < 5$ %)

Table 2.12 Effects of phosphorus sources on shoot dry weight (g $plant^{-1}$) of silage corn genotypes at black layer stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
2016	P_0	112.5 ^{k}	133.7 ^{fghij}	98.67 ^{l}	126.33 ^{j}	129.67 ^{ghij}	120.17 ^{D}
	P_1	146.67 ^{bcde}	169.17 ^{a}	130.67 ^{ghij}	147.17 ^{bcd}	151 ^{b}	148.93 ^{A}
	P_2	145.7 ^{bcde}	149.33 ^{bc}	126.87 ^{ij}	130.33 ^{ghij}	143.67 ^{bcdef}	139.18 ^{B}
	P_3	138.17 ^{defgh}	136.67 ^{efghi}	125.77 ^{j}	129.17 ^{hij}	139.37 ^{cdefg}	133.83 ^{C}
	Means	135.76 ^{C}	147.22 ^{A}	120.49 ^{D}	133.25 ^{C}	140.93 ^{B}	
2017	P_0	92.44	117.56	89.11	72.33	89.11	92.11 ^{C}
	P_1	130.44	138.56	115.67	88.22	147.22	124.02 ^{A}
	P_2	127	136.11	108.22	85.22	103.33	111.98 ^{B}
	P_3	107	129.22	94.44	80	95.11	101.16 ^{BC}
	Means	114.22 ^{B}	130.36 ^{A}	101.86 ^{B}	81.44 ^{C}	108.69 ^{B}	

(Common letter means no significant difference at $p < 5$ %)

Table 2.13 Effects of phosphorus sources on root shoot ratio of silage corn genotypes at tasseling stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC23-17RIB	DKC26-28RIB	Means
2016	P_0	0.83 ^{bcd}	0.90 ^{ab}	0.96 ^{a}	0.92 ^{ab}	0.94 ^{a}	0.91 ^{A}
	P_1	0.69 ^{fghi}	0.72 ^{efg}	0.66 ^{fghij}	0.58 ^{jk}	0.60 ^{ijk}	0.65 ^{C}
	P_2	0.73 ^{efgh}	0.89 ^{abc}	0.75 ^{defg}	0.73 ^{efgh}	0.80 ^{cde}	0.78 ^{B}
	P_3	0.76 ^{def}	0.61 ^{ijk}	0.64 ^{hijk}	0.65 ^{ghij}	0.55 ^{k}	0.64 ^{C}
	Means	0.75 ^{AB}	0.78 ^{A}	0.75 ^{AB}	0.72 ^{B}	0.72 ^{B}	
2017	P_0	0.31 ^{a}	0.23 ^{bcdef}	0.24 ^{abcd}	0.23 ^{bcdef}	0.24 ^{bcde}	0.25 ^{A}
	P_1	0.17 ^{efg}	0.16 ^{fg}	0.16 ^{fg}	0.22 ^{bcdef}	0.24 ^{abcd}	0.19 ^{B}
	P_2	0.17 ^{defg}	0.17 ^{efg}	0.19 ^{cdefg}	0.28 ^{ab}	0.22 ^{bcdef}	0.21 ^{B}
	P_3	0.18 ^{defg}	0.15 ^{g}	0.21 ^{bcdefg}	0.28 ^{ab}	0.26 ^{abc}	0.22 ^{B}
	Means	0.21 ^{BC}	0.18 ^{C}	0.20 ^{C}	0.25 ^{A}	0.24 ^{AB}	

(Common letter means no significant difference at $p < 5$ %)

Table 2.14 Effects of phosphorus sources on plant height (cm^2) of silage corn genotypes at black layer stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
2016	P_0	208 ^{ij}	218.67 ^{gh}	188.33 ^{m}	218.33 ^{gh}	227.4 ^{bcdef}	212.15 ^{C}
	P_1	225.53 ^{cdefg}	236.33 ^{a}	204.37 ^{jk}	229.33 ^{abcde}	232.33 ^{abc}	225.58 ^{A}
	P_2	222.67 ^{defgh}	233.67 ^{ab}	197 ^{kl}	222 ^{efgh}	223.26 ^{defgh}	219.72 ^{B}
	P_3	221 ^{fgh}	228.66 ^{abcdef}	193.33 ^{lm}	215.8 ^{hi}	230.27 ^{abcd}	217.81 ^{B}
	Means	219.3 ^{B}	229.33 ^{A}	195.76 ^{C}	221.37 ^{B}	228.31 ^{A}	
2017	P_0	161.61	184.61	148.61	154.11	171.89	164.17 ^{C}
	P_1	187.06	206.17	152.78	171.67	197.5	183.03 ^{A}
	P_2	179.06	196	150.22	166.67	188.06	176 ^{AB}
	P_3	178.22	192.72	149.94	160.17	175.5	171.31 ^{B}
	Means	176.49 ^{B}	194.87 ^{A}	150.39 ^{D}	163.15 ^{C}	183.24 ^{B}	

(Common letter means no significant difference at $p < 5$ %)

Table 2.15 Effects of phosphorus sources on dry matter yield (Mg ha^{-1}) of silage corn genotypes at black layer stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
2016	P_0	14.65 ^{i}	14.98 ^{hi}	13.12 ^{j}	15.07 ^{ghi}	16.24 ^{efgh}	14.81 ^{D}
	P_1	19.17 ^{c}	21.94 ^{a}	17.37 ^{de}	17.31 ^{de}	19.13 ^{c}	18.98 ^{A}
	P_2	16.45 ^{efg}	20.75 ^{ab}	15.67 ^{fghi}	16.07 ^{efghi}	18.53 ^{cd}	17.49 ^{B}
	P_3	16.33 ^{efgh}	19.95 ^{bc}	14.66 ^{i}	15.77 ^{fghi}	16.61 ^{ef}	16.66 ^{C}
	Means	16.65 ^{C}	19.40 ^{A}	15.21 ^{D}	16.06 ^{C}	17.63 ^{B}	
2017	P_0	10.1	13.77	10.09	7.54	11.84	10.67 ^{C}
	P_1	15.46	16.87	12.29	11.14	15.4	14.23 ^{A}
	P_2	12.88	15.57	11.47	10.46	13.01	12.68 ^{B}
	P_3	12.19	13.76	10.23	8.23	11.64	11.21 ^{C}
	Means	12.66 ^{B}	14.99 ^{A}	11.02 ^{C}	9.34 ^{D}	12.97 ^{B}	

(Common letter means no significant difference at $p < 5$ %)

Acid phosphatase (Ap-ase) activity was significantly (*p<0.001*) influenced with interactive effects of G × P sources during 2016 (Table 2.16) and highest Ap-ase activity of 19.07 µmole PNP g^{-1} min^{-30} was observed when Yukon R was amended with high P manure, followed by DKC23-17RIB and DKC26-28RIB under same treatment, whereas lowest AP-ase activity was found in control treatment in the rhizosphere of Fusion RR (Table 2.19). In 2017, G x P interaction had non-significant effects on Ap-ase activity (Table 2.17). Individual comparison of treatments showed that high Ap-ase activity of 83.45 µmole PNP g^{-1} min^{-30} was observed in high P manure treatment, and lowest Ap-ase (58.08 µmole PNP g^{-1} min^{-30}) was recorded in control treatment (Table 19). Genotypes had non-significant (*p = 0.08*) effects on Ap-ase activity (Table 2.17).

Soil available phosphorus (SAP) was significantly (*p<0.01*) affected by G × P sources interaction during 2016 (Table 2.16). Higher SAP of 129.36 mg/kg was noted when high P manure was applied to Yukon R, and lowest SAP (49.67 mg/kg) was found in the rhizosphere of A4177G3RIB genotype in the control treatment. In 2017 growing season, G × P sources had non-significant effects on SAP (Table 2.17). Genotypes and P sources as individual factor had significantly affected SAP. Among genotypes Yukon R showed highest SAP (128.29 mg/kg) in soil rhizosphere while the lowest was found in the soil rhizosphere of DKC23-17RIB (Table 2.20). Among P sources, both manure sources either with high and low P concentration showed superior performance and provided higher SAP compared to control treatment which showed lowest SAP. Interestingly, IP and control treatments were statistically at par with each other and were similar in their response to SAP (Table 2.20).

2.4.3. Soil PLFA profiles

Analysis of variance showed that G × P sources interaction had non-significant effects on soil microbial community during 2016. Genotypes as individual factor had significantly affected the Gram positive (G^+), Gram negative (G^-) bacterial population, fungi, eukaryotes, total active bacterial PLFAs biomass (Σ B-PLFAS), total active PLFA biomass (Σ PLFAS) and G^+:G^- and non-significantly affected protozoa and fungi-bacteria ratio (F:B). Among genotypes, Yukon R had the maximum active soil microbial biomass in the root rhizospheres except G^+: G^- ratio. However, genotypes had non-significant effects on Protozoa and F:B. Yukon R and DKC26-28RIB were statistically at par in producing total active bacterial PLFAs biomass. P sources had significantly affected G^-, fungi, Σ B-PLFAS and Σ PLFAS and non-significant effects on G+, protozoa, eukaryotes, G^+: G^- and F:B. In 2017, Genotypes × P sources had also non-significantly affected PLFAs except fungi and F:B (Table 2.22). Fungi was significantly ($p<0.01$) influenced by G × P sources, and highest fungal PLFAs biomass was noted in the rhizosphere of Yukon R under high P manure application (Table 2.25). Genotypes had significantly affected all active microbial communities biomass except protozoa, eukaryotes, and G^+: G^-. Among genotypes, overall, high values of active bacterial PLFAs biomass and total PLFAs was exhibited by Yukon R (Table 2.23). However, protozoa and G^+: G^- ratio was statistically at par in all genotypes. P sources had significantly affected G-, fungi, total active bacterial PLFAs biomass and total PLFAs, and non-significant effects were observed for G+, protozoa, eukaryotes, G^+: G^- ratio and F:B ratio during 2016 (Table 2.21). Higher G^- bacterial population was noted when high P manure was applied and lowest was noted in inorganic P source. Similarly,

maximum active fungal PLFAs biomass was recorded in high P manure compared to minimum active fungal PLFAs biomass in control treatment. Higher eukaryotes, total active bacterial PLFAs biomass and total PLFAs were also observed in high P manure treatment. In 2017, P sources had significantly affected the active microbial composition except for the protozoa and G^+/G^- ratio. High P manure source produced maximum G+, G-, fungi, eukaryotes, total active bacterial PLFA biomass, and total active microbial PLFA biomass followed by low P manure source (Table 2.24). It appears that manure with either high or low P source enhanced PLFAs compared to inorganic P source and control treatment. During both years (2016 & 2017), Yukon R genotype exhibited significantly higher G^+ bacteria followed by DKC26-28RIB and Fusion RR, and lowest G^+ bacteria was found in the rhizosphere of A4177G3RIB and DKC23-17RIB (Table 2.23). Manure application with high and low P concentration significantly increased G^+ community as compared to inorganic P and control (Table 2.24).

As shown in Table 2.21 & 2.22, silage corn genotypes and phosphorus sources significantly affected Gram negative bacteria, however, their interaction between genotypes and phosphorus sources were not significant. During 2016 and 2017, Yukon R had the higher G^- bacteria in their rhizosphere followed by DKC26-28RIB, and the lowest was found in Fusion RR and DKC23-17RIB (Table 2.23). High P manure source significantly enhanced G^- bacteria compared to inorganic P and control. High G^- bacterial population was 22.94 and 23.10 nmol g^{-1} during 2016 and 2017 (Table 2.24)

Table 2.16 ANOVA for biochemical parameters at black layer stage during 2016

Biochemical Parameters	Genotypes	Phosphorus sources	G x P	Coefficient of variation%
Soil pH	***	*	*	0.90
Acid phosphatase activity	***	***	***	2.72
Soil available P	***	***	**	8.07

Table 2.17 ANOVA for biochemical parameters at black layer stage during 2017

Biochemical parameters	Genotypes	Phosphorus sources	G x P	Coefficient of variation%
Soil pH	***	**	**	2.78
Acid phosphatase activity	NS	***	NS	21.73
Soil available P	***	***	NS	21.37

(*** Significant at $p < 0.001$, ** Significant at $p < 0.1$, *Significant at $p < 0.05$, NS= Non-Significant)

Table 2.18 Effects of phosphorus sources on soil pH at black layer stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
2016	P_0	6.09 ^{ab}	6.09 ^{ab}	5.92 ^{efg}	5.98 ^{cdef}	5.98 ^{cdef}	6.01 ^{A}
	P_1	6.006 ^{bcde}	6.06 ^{abc}	5.96 ^{defg}	5.95 ^{defg}	5.96 ^{defg}	5.99 ^{AB}
	P_2	6.03 ^{abcd}	6.01 ^{bcd}	5.95 ^{defg}	5.89 ^{fgh}	5.98 ^{cdef}	5.97 ^{AB}
	P_3	6.12 ^{a}	6.02 ^{bcd}	5.92 ^{efg}	5.88 ^{gh}	5.83 ^{h}	5.95 ^{B}
	Means	6.06 ^{A}	6.04 ^{A}	5.94 ^{B}	5.92 ^{B}	5.94 ^{B}	
2017	P_0	6.18 ^{abcde}	6.05 ^{bcdefg}	5.91 ^{efgh}	5.58 ^{i}	5.73 ^{hi}	5.89 ^{C}
	P_1	5.88 ^{fgh}	6.29 ^{ab}	6.28 ^{abc}	6.20 ^{abcd}	6.11 ^{abcdef}	6.15 ^{A}
	P_2	6.10 ^{abcdef}	5.97 ^{defgh}	6.06 ^{bcdef}	5.82 ^{ghi}	5.93 ^{defgh}	5.97 ^{BC}
	P_3	6.08 ^{bcdefg}	6.13 ^{abcdef}	6.38 ^{a}	5.57 ^{i}	6.00 ^{cdefgh}	6.03 ^{AB}
	Means	6.06 ^{AB}	6.11 ^{A}	6.15 ^{A}	5.79 ^{C}	5.94 ^{B}	

(Common letter means no significant difference at $p < 5$ %)

Table 2.19 Effects of phosphorus sources on acid phosphatase activity (µmole PNP g^{-1} min^{-30}) at black layer stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
2016	P_0	14.55 ^{hij}	14.99 ^{fgh}	13.95 ^{j}	11.03 ^{k}	14.25 ^{ij}	13.75 ^{C}
	P_1	17.15 ^{cd}	19.07 ^{a}	16.70 ^{d}	17.85 ^{bc}	17.91 ^{b}	17.73 ^{A}
	P_2	15.54 ^{ef}	16.94 ^{d}	15.30 ^{efg}	15.68 ^{ef}	15.93 ^{e}	15.88 ^{B}
	P_3	14.48 ^{hij}	14.83 ^{ghi}	14.70 ^{ghi}	18.21 ^{b}	15.84 ^{e}	15.61 ^{B}
	Means	15.43 ^{CD}	16.46 ^{A}	15.16 ^{D}	15.69 ^{BC}	15.98 ^{B}	
2017	P_0	40.80	62.96	62.18	68.58	55.88	58.08 C
	P_1	78.71	95.07	86.29	76.75	80.47	83.45 A
	P_2	67.30	94.49	81.04	75.09	70.30	77.64 AB
	P_3	67.75	69.54	74.71	69.36	62.97	68.86 BC
	Means	63.64 ^{B}	80.51 ^{A}	76.05 ^{AB}	72.44 ^{AB}	67.40 ^{B}	

(Common letter means no significant difference at $p < 5$ %)

Table 2.20 Effects of phosphorus sources on soil available P (mg/kg) at black layer stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
2016	P_0	63.9 ^{hi}	74.59 ^{efg}	49.67 ^{j}	56.59 ^{ij}	57.95 ^{ij}	60.54 ^{D}
	P_1	93.65 ^{cd}	129.36 ^{a}	77.61 ^{efg}	83.26 ^{de}	100.78 ^{bc}	96.93 ^{A}
	P_2	75.82 ^{efg}	107.79 ^{b}	69.66 ^{fgh}	78.31 ^{efg}	96.45 ^{c}	85.60 ^{B}
	P_3	69.01 ^{gh}	93.67 ^{cd}	52.25 ^{j}	68.97 ^{gh}	80.03 ^{ef}	72.78 ^{C}
	Means	75.59 ^{C}	101.35 ^{A}	62.3 ^{D}	71.78 ^{C}	83.8 ^{B}	
2017	P_0	73.12	119.59	66.62	49.86	67.34	75.3 ^{B}
	P_1	112.92	139.75	88.17	67.05	116.9	104.96 ^{A}
	P_2	100.34	132.7	78.22	74.72	114.26	100.05 ^{A}
	P_3	81.99	121.1	72.61	55.09	57.08	77.57 ^{B}
	Means	92.09 ^{B}	128.29 ^{A}	76.41 ^{BC}	61.68 ^{C}	88.89 ^{B}	

(Common letter means no significant difference at $p < 5$ %)

Table 2.21 ANOVA for soil microbial communities at black layer stage during 2016

Soil microbial community	Genotypes	Phosphorus sources	G x P	Coefficient of variation%
G+	*	NS	NS	13.65
G-	***	***	NS	6.97
Fungi	*	*	NS	14.02
Protozoa	NS	NS	NS	13.21
Eukaryotes	*	NS	NS	13.27
Total Bacterial PLFAs	***	*	NS	8.47
Total PLFAs	***	**	NS	7.47
G+:G-	***	NS	NS	13.97
F:B	NS	NS	NS	19.64

(*** Significant at $p < 0.001$, ** Significant at $p < 0.1$, *Significant at $p < 0.05$, NS= Non-Significant)

Table 2.22 ANOVA for soil microbial communities at black layer stage during 2017

Soil microbial community	Genotypes (G)	Phosphorus sources (P)	G x P	Coefficient of variation%
G+	**	**	NS	5.22
G-	***	***	NS	4.67
Fungi	***	***	**	12.2
Protozoa	NS	NS	NS	9.19
Eukaryotes	NS	***	NS	30.47
Total Bacterial PLFAs	***	***	NS	3.94
Total PLFAs	***	***	NS	3.77
G+:G-	NS	NS	NS	5.79
F:B	*	***	**	13.2

(*** Significant at $p < 0.001$, ** Significant at $p < 0.1$, *Significant at $p < 0.05$, NS= Non-Significant)

Table 2.23 Effects of silage corn genotypes on PLFAs (nmol g^{-1} soil) at black layer stage during 2016 and 2017

	Genotypes	G+	G-	F	P	E	Σ B-PLFAs	Σ PLFAs	G^+:G^-	F:B
2016	Fusion RR	20.77 ^{ab}	19.00 ^{d}	3.56 ^{bc}	1.48 ^{a}	2.56 ^{ab}	39.77 ^{b}	47.39 ^{c}	1.09 ^{a}	0.09 ^{a}
	Yukon R	21.74 ^{a}	24.76 ^{a}	4.02 ^{a}	1.55 ^{a}	2.78 ^{a}	46.50 ^{a}	54.87 ^{a}	0.88 ^{b}	0.086 ^{a}
	A4177G3RIB	18.04 ^{c}	20.59 ^{c}	3.26 ^{c}	1.39 ^{a}	2.39 ^{b}	38.63 ^{b}	45.69 ^{c}	0.87 ^{b}	0.087 ^{a}
	DKC 23-17RIB	18.81 ^{bc}	21.19 ^{c}	3.63 ^{abc}	1.54 ^{a}	2.75 ^{a}	40.00 ^{b}	47.93 ^{c}	0.89 ^{b}	0.090 ^{a}
	DKC 26-28RIB	20.85 ^{ab}	22.89 ^{b}	3.74 ^{ab}	1.50 ^{a}	2.77 ^{a}	43.75 ^{a}	51.76 ^{b}	0.91 ^{b}	0.085 ^{a}
2017	Fusion RR	21.04 ^{a}	22.05 ^{ab}	3.34 ^{bc}	1.72 ^{a}	1.72 ^{ab}	43.09 ^{ab}	49.89 ^{bc}	0.95 ^{a}	0.077 ^{ab}
	Yukon R	21.43 ^{a}	22.85 ^{a}	3.69 ^{a}	1.79 ^{a}	1.91 ^{a}	44.29 ^{a}	51.69 ^{a}	0.93 ^{a}	0.083 ^{a}
	A4177G3RIB	20.98 ^{a}	21.60 ^{bc}	3.13 ^{cd}	1.81 ^{a}	1.48 ^{b}	42.58 ^{b}	49.00 ^{c}	0.97 ^{a}	0.073 ^{b}
	DKC 23-17RIB	19.74 ^{b}	20.84 ^{c}	2.86 ^{d}	1.79 ^{a}	1.45 ^{b}	40.58 ^{c}	46.71 ^{d}	0.95 ^{a}	0.070 ^{b}
	DKC 26-28RIB	21.25 ^{a}	22.64 ^{a}	3.46 ^{ab}	1.71 ^{a}	1.98 ^{a}	43.89 ^{ab}	51.06 ^{ab}	0.94 ^{a}	0.079 ^{ab}

Note: Abbreviations: G+ = Gram positive, G- = Gram negative, F = Fungi, P = Protozoa, E = Eukaryotes, Σ B-PLFAs = Total Bacterial PLFAs, Σ PLFAs = Total PLFAs, G+: G- = Gram positive/Gram negative ratio, F: B = Fungi/Bacteria ratio.
(Common letter means does not differ significantly at $p < 5$ %)

Table 2.24 Effects of phosphorus sources on PLFAs (nmol g^{-1} soil) at black layer stage during 2016 and 2017

	P sources	G+	G-	F	P	E	Σ B-PLFAs	Σ PLFAs	G^+:G^-	F:B
2016	P_0	19.764 ^{a}	21.262 ^{bc}	3.390 ^{b}	1.421 ^{a}	2.471 ^{b}	41.026 ^{ab}	48.309 ^{bc}	0.938 ^{a}	0.082 ^{a}
	P_1	20.365 ^{a}	22.942 ^{a}	3.975 ^{a}	1.509 ^{a}	2.812 ^{a}	43.307 ^{a}	51.605 ^{a}	0.883 ^{a}	0.094 ^{a}
	P_2	20.795 ^{a}	22.024 ^{ab}	3.725 ^{ab}	1.545 ^{a}	2.720 ^{ab}	42.819 ^{a}	50.81 ^{ab}	0.954 ^{a}	0.087 ^{a}
	P_3	19.259 ^{a}	20.533 ^{c}	3.487 ^{b}	1.515 ^{a}	2.617 ^{ab}	39.793 ^{b}	47.412 ^{c}	0.947 ^{a}	0.087 ^{a}
2017	P_0	20.698 ^{bc}	21.555 ^{b}	2.691 ^{c}	1.809 ^{a}	1.394 ^{c}	42.253 ^{bc}	48.149 ^{c}	0.960 ^{a}	0.063 ^{c}
	P_1	21.618 ^{a}	23.101 ^{a}	3.778 ^{a}	1.776 ^{a}	2.373 ^{a}	44.719 ^{a}	52.647 ^{a}	0.938 ^{a}	0.084 ^{a}
	P_2	21.076 ^{ab}	22.003 ^{b}	3.700 ^{a}	1.730 ^{a}	1.952 ^{b}	43.079 ^{b}	50.462 ^{b}	0.958 ^{a}	0.086 ^{a}
	P_3	20.182 ^{c}	21.335 ^{b}	3.025 ^{b}	1.763 ^{a}	1.127 ^{c}	41.517 ^{c}	47.434 ^{c}	0.948 ^{a}	0.072 ^{b}

(Common letter means no significant difference at $p < 5$ %)

Table 2.25 Effects of phosphorus sources on fungal PLFAs (nmol g^{-1} soil) at black layer stage during 2016 and 2017

	G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
2016	P_0	3.49	3.64	2.39	3.57	3.87	3.39 ^{B}
	P_1	3.74	4.70	3.52	4.02	3.89	3.97 ^{A}
	P_2	3.55	3.91	3.69	3.55	3.92	3.72 ^{AB}
	P_3	3.50	3.83	3.44	3.39	3.28	3.48 ^{B}
	Means	3.56 ^{BC}	4.02 ^{A}	3.26 ^{C}	3.63 ^{ABC}	3.74 ^{AB}	
2017	P_0	2.00 ^{e}	3.42 ^{bc}	2.07 ^{e}	2.61 ^{de}	3.34 ^{c}	2.69 ^{C}
	P_1	4.07 ^{ab}	4.08 ^{a}	3.59 ^{abc}	3.46 ^{abc}	3.67 ^{abc}	3.77 ^{A}
	P_2	4.06 ^{ab}	4.06 ^{ab}	3.59 ^{abc}	3.21 ^{cd}	3.56 ^{abc}	3.70 ^{A}
	P_3	3.23 ^{cd}	3.17 ^{cd}	3.25 ^{cd}	2.18 ^{e}	3.27 ^{cd}	3.02 ^{B}
	Means	3.34 ^{BC}	3.69 ^{A}	3.13 ^{CD}	2.86 ^{D}	3.46 ^{AB}	

(Common letter means no significant difference at $p < 5$ %)

Table 2.26 Effects of phosphorus sources on Fungi: Bacteria (F: B) ratio at black layer stage during 2017

G x P	Fusion RR	Yukon R	A4177G3RIB	DKC 23-17RIB	DKC26-28RIB	Means
P_0	0.047 ^{e}	0.078 ^{abc}	0.049 ^{de}	0.065 ^{cd}	0.078 ^{abc}	0.063 ^{C}
P_1	0.091 ^{ab}	0.089 ^{ab}	0.081 ^{abc}	0.081 ^{abc}	0.080 ^{abc}	0.084 ^{A}
P_2	0.094 ^{a}	0.091 ^{ab}	0.084 ^{ab}	0.078 ^{abc}	0.080 ^{abc}	0.086 ^{A}
P_3	0.077 ^{bc}	0.075 ^{bc}	0.078 ^{abc}	0.057 ^{de}	0.076 ^{bc}	0.072 ^{B}
Means	0.077 ^{AB}	0.083 ^{A}	0.073 ^{B}	0.070 ^{B}	0.079 ^{AB}	

(Common letter means no significant difference at $p < 5$ %)

2.4.3.1. Principal Component Analysis (PCA)

Bi-plot showed the relationship between the PLFAs silage corn genotypes and P sources applied during both growing seasons (Figure 2.4.1 & 2.4.2). PC1 and PC2 accounted for 40.81% and 26.31% of the total variation observed in the data. Biochemical attributes and PLFAs showed a significant separation of Yukon R and DKC26-28RIB from other genotypes (Figure 2.4.1a) and high and low P manure from other P sources (Figure 2.4.1b) in 2016. In 2017, PC1 and PC2 accounted for 40.92% and 18.37% of the total variation in the data. Yukon R and DKC26-28RIB showed clear separation with enzyme activity, available P and PLFAs compared to other genotypes (Figure 2.4.2a). Soil biochemical attributes and microbial communities also showed a significant separation with P_1 and P_2 and other P sources (Figure 2.4.2b). A clear separation was also found when comparing the soil biochemical parameters and microbial communities of P_3 and P_0 along the PC2 axis (Figure 2.4,2b). PCA indicates that manure applications with high and low P concentration significantly improved soil biochemical attributes and the active microbial biomass or diversity. A positive association between Yukon R, DKC26-28RIB, PLFA parameters and biochemical attributes was observed. Similarly, positive correlation was noted between P_1 and P_2 sources, PLFAs and soil biochemical attributes (Figure 2.4.1b & 2.4.2b). Further confirmation of these relationships was demonstrated by Pearson correlation analysis between biochemical attributes and PLFAs (Table 2.28).

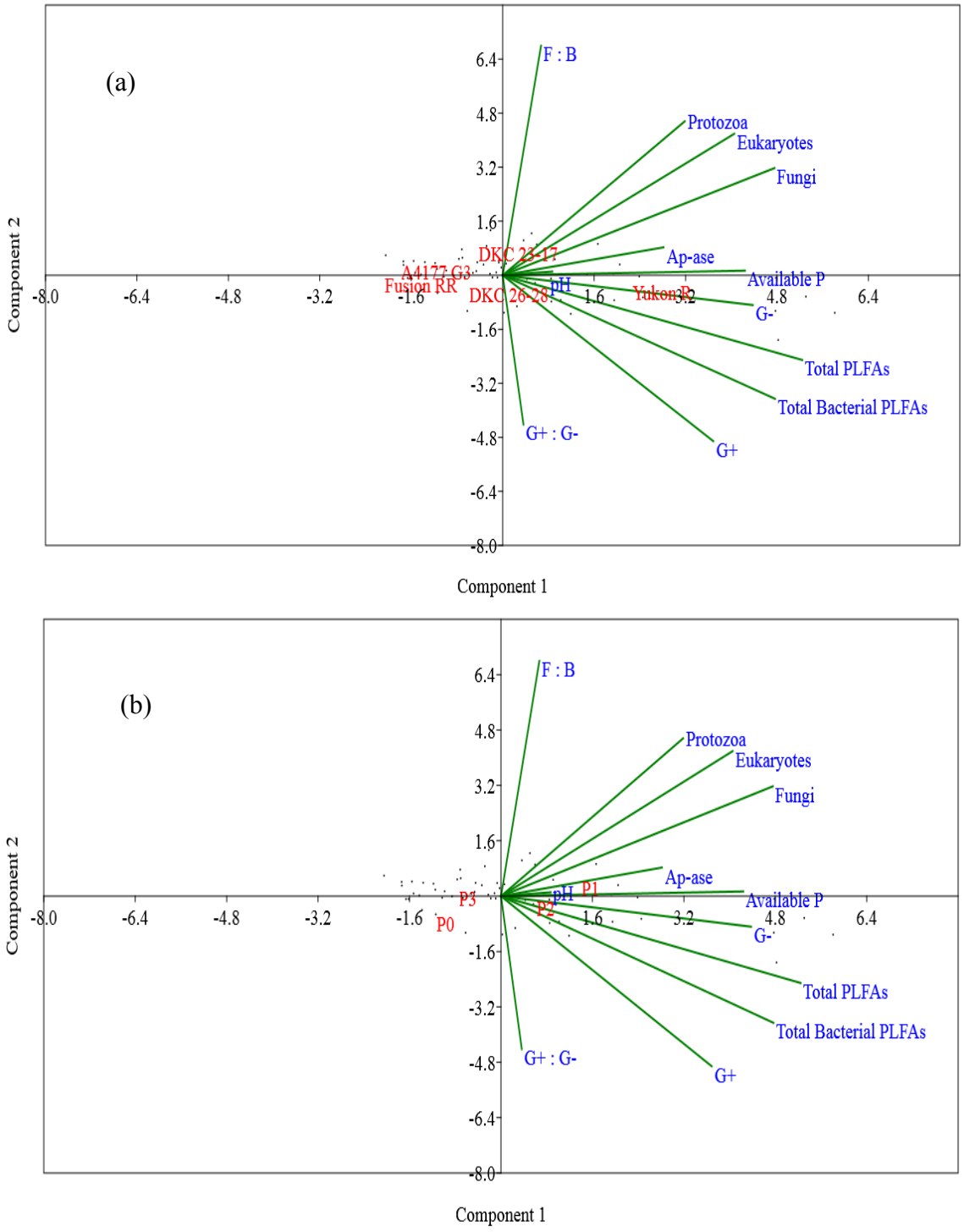

Figure 2.4.1 Phosphorus sources and silage corn genotypes alter biochemical attributes and active microbial communities under field conditions. Bi-plot showing relationships between observed biochemical attributes, active microbial community composition and abundance in (a) silage corn genotypes (b) P sources during 2016.

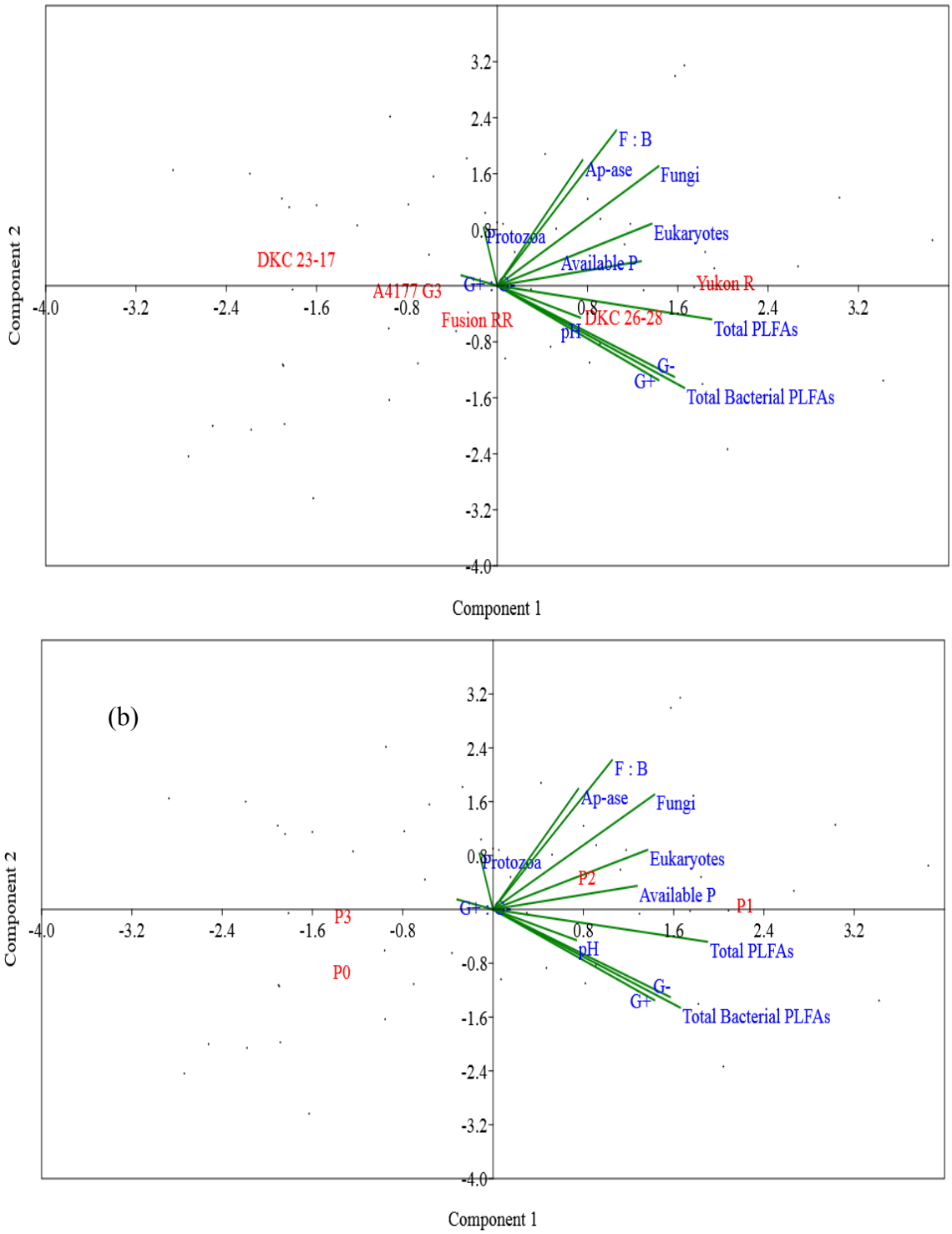

Figure 2.4.2 Phosphorus sources and silage corn genotypes alter biochemical attributes and active microbial communities under field conditions. Bi-plot showing relationships between observed biochemical attributes, active microbial community composition and abundance in (a) silage corn genotypes (b) P sources during 2017.

2.4.3.2. Pearson's correlation between agronomic parameters, soil biochemical attributes and microbial community composition

During 2016 and 2017, the agronomic parameters (leaf area, chlorophyll contents, photosynthesis rate, plant height and shoot dry weight) showed significant relationships with dry matter yield (Table 2.27). Positive and very strong relationships was noticed between LA and DMY, photosynthesis rate (PR) and DMY. Pearson correlation analysis between biochemical parameters (Ap-ase, SAP and pH) and PLFAs are given in Table 2.28. In 2016, soil pH showed non-significant relationship with Ap-ase, SAP and soil microbial communities. Whereas during 2017, significant relationship of soil pH with SAP, G+, G-, total bacterial PLFAs and total PLFAs was observed. Positive and strong correlations were found between SAP, Ap-ase, fungi, bacterial PLFA and total PLFA abundance (Table 2.28). Microbial community was significantly correlated with Ap-ase in 2016, while bacterial and total PLFA abundance showed significant relationship with Ap-ase activity during 2017.

Table 2.27 Pearson's correlation coefficients (r) showing the relationship between agronomic performance parameters and dry matter yield during 2016 and 2017

Years		LA	Chl	Pr	PH	SDW
2016	Chl	0.71***				
	Pr	0.83***	0.79***			
	PH	0.72***	0.56***	0.72***		
	SDW	0.81***	0.69***	0.79***	0.76***	
	DMY	0.80***	0.77***	0.85***	0.66***	0.79***
2017	Chl	0.63***				
	Pr	0.51***	0.54***			
	PH	0.72***	0.43***	0.51***		
	SDW	0.67***	0.49***	0.52***	0.60***	
	DMY	0.75***	0.58***	0.67***	0.67***	0.64***

Note: Abbreviations: LA = Leaf area, Chl = Chlorophyll contents, Pr = Photosynthesis rate, PH = Plant height, SDW = Shoot dry weight, DMY = Dry matter yield.
(*** Significant at $p < 0.001$, ** Significant at $p < 0.1$, *Significant at $p < 0.05$, NS= Non-Significant).

Table 2.28 Pearson's correlation coefficients (r) showing the relationship between soil biochemical parameters and active microbial communities during 2016 and 2017

Years		Ap-ase	SAP	pH
2016	Ap-ase		0.68***	-0.09^{Ns}
	SAP			0.19^{Ns}
	G+	0.17^{Ns}	0.36**	0.12^{Ns}
	G-	0.27*	0.57***	-0.00^{Ns}
	F	0.35**	0.51***	0.21^{Ns}
	P	0.11^{Ns}	0.21^{Ns}	0.04^{Ns}
	E	0.31*	0.40**	0.04^{Ns}
	ΣB-PLFAs	0.26*	0.55***	0.07^{Ns}
	ΣPLFAs	0.30*	0.59***	0.09^{Ns}
	G+:G-	-0.05^{Ns}	-0.09^{Ns}	0.13^{Ns}
	F:B	0.19^{Ns}	0.11^{Ns}	0.14^{Ns}
2017	Ap-ase		0.30*	0.10^{Ns}
	SAP			0.28*
	G+	-0.01^{Ns}	0.39**	0.27*
	G-	0.08^{Ns}	0.39**	0.30*
	F	0.48***	0.48***	0.15^{Ns}
	P	0.10^{Ns}	-0.09^{Ns}	0.03^{Ns}
	E	0.44***	0.39**	0.08^{Ns}
	ΣB-PLFAs	0.04^{Ns}	0.43***	0.32*
	ΣPLFAs	0.25^{Ns}	0.52***	0.30*
	G+:G-	-0.10^{Ns}	-0.04^{Ns}	-0.06^{Ns}
	F:B	0.49***	0.38**	0.08^{Ns}

Note: Abbreviations: Ap-ase = Acid phosphatase, SAP = Soil available P, G+ = Gram positive, G- = Gram negative, F = Fungi, P = Protozoa, E = Eukaryotes, Σ B-PLFAs = Total Bacterial PLFAs, Σ PLFAs = Total PLFAs, G+: G- = Gram positive/Gram negative ratio, F: B = Fungi/Bacteria ratio.
(*** Significant at $p < 0.001$, ** Significant at $p < 0.1$, *Significant at $p < 0.05$, NS= Non-Significant)

2.5. Discussion

2.5.1. Agronomic performance parameters

2.5.1.1. Leaf area

Leaves play very important roles in photosynthesis and other physiological processes which leads to enhance dry biomass (Khan and Khalil, 2010; Man et al., 2015). DM with high and low P significantly increased LA of silage corn genotypes compared to control (Table 2.9). Phosphorus plays very exclusive role in cell division (Kavanova et al., 2006) and cell elongation in crop plants (Radin and Eidenbock, 1984). Phosphate availability in leaves increased expansion of epidermal cells and consequently leaf blade (Waldron and Terry, 1987) and phosphate concentration in the upper epidermis cells rapidly decreased with decreased P supply to the leaf (Treeby et al., 1987). LA of corn significantly increased with DM application compared to control and this increment was attributed to optimum and consistent supply of N and P nutrients to the plant (Hariadi et al., 2016). In 2016, G × P sources interaction showed significant increase in LA of Yukon R under high P manure treatment. The observed increment in LA of Yukon R might be related to higher P-uptake genetic potential or high P manure treatment enhanced P availability which improved cell division and elongation process led to higher LA development in Yukon R compared to other genotypes. In dry season or in arid conditions, reduced soil moisture can reduce P uptake and availability in epidermal cells and consequently reduction in LA of silage corn. Previous study conducted by Song et al., (2018) reported that soil moisture deficit significantly limit vegetative growth, lead to

decline in LA of corn. Our results of 2017 growing season are in agreement with that of Song et al., (2018).

2.5.1.2. Chlorophyll contents

Chlorophyll contents in crop plants are important in energy harvesting reaction during photosynthesis process that help in CO_2 fixation and maximization of carbohydrates for energy production (Slamet et al., 2017). Chlorophyll is part of the chloroplast, and predominantly used inorganic P (Pi) (Giesrsch and Robinson, 1987), and deficiency of P supply to chloroplast can inhibits photosynthesis (Dietz and Foyer, 1986). Light, carbohydrates, temperature, plant species and genetic potential, and nutrients availability to crop plants may affects the chlorophyll synthesis and photosynthesis rate (Hasan et al., 2014). DM and other organic fertilizers provide essential nutrients for plant growth and have a positive effect on the formation of chlorophyll and chloroplast (Hasan et al., 2014). Organic acids either released by plant roots or produced during the decomposition and mineralization of manure/organic matter play an important role in gradual and consistent supply of Mg^{+2} which contribute to the formation of chlorophyll molecules compared to inorganic fertilizers (Hasan et al., 2014). Organic fertilizer sources increased the availability and absorption of essential elements, such as Fe^{2+}, Mg^{2+} and NH^{4+}, necessary for enzyme activation, chloroplast and chlorophyll formation (Elhindi, 2012). DM application significantly increased chlorophyll contents of maize compared to control (Efthimiadou et al., 2009). In present study, we found that DM application either with high/low P significantly increased chlorophyll contents of Yukon R and DKC26-28RIB

compared to control over the both growing season (Table 2.10). Increased chlorophyll contents with high/low manure P treatments, suggest that optimum supply of N, P and Mg^{+2} to crop plants might have increased chlorophyll contents because both compounds are central component of chlorophyll molecules. Our results are in agreement with previous findings (Elhindi, 2012) who reported that manure application significantly improved chlorophyll contents of crop plants presumably by increasing availability of N and Mg^{2+} in soil. Chlorophyll contents also showed positive relationship with photosynthesis rate in crops. (Efthimiadou et al., 2009; Reich et al., 1994).

2.5.1.3. Photosynthesis rate

Photosynthesis is an important determinant of growth and yield of crops (Murchie et al., 2008). Phosphorus availability facilitate supply of triose-P from the chloroplast to the cytosol via the Pi translocator (Giersch and Robinson, 1987; Wissuwa et al., 2005). Phosphorus is an important element in compounds such as ATP, NADPH, nucleic acids, sugar phosphates, and phospholipids which involves in photosynthesis (Hammond and White, 2008). Photosynthesis is a process in which plants absorbed light energy and convert in to chemical energy (Hohmann-Marriott and Blankenship, 2011). P deficient plants showed reduction in photosynthesis that might be due to diminishing of ribulose 1-5, bisphosphate (RuBP) generation rather than due to limitations in the supply of ATP (adenosine triphosphate) and NADPH (Nicotinamide adenine dinucleotide phosphate oxidase) in the Calvin cycle (Fredeen et al., 1989). RuBP is formed by taking a phosphate coming from the splitting of ATP, and joining it with riblulose phosphate (RuP), changing

RuP into RuBP, which is then able to react with CO_2 and form an unstable 6C (carbon) molecule which is the basis for the dark reaction. ATP is commonly known as energy currency of the cell because energy obtained through its metabolism is used for biomolecules synthesis, movement and cell division. ATP consists of the purine base adenine, pentose sugar ribose, and three phosphate groups, and anhydrous bonds of the three phosphate make ATP a high energy molecule. During the photosynthetic electron transfer reaction, H^+ pumped across the thylakoid membrane, and the resulting electrochemical proton gradient derives the synthesis of ATP in the stroma (Alberts et al., 2002). APT hydrolysis produces more free energy compounds such as ADP (adenosine diphosphate) and inorganic phosphate than that of other phosphate compounds and common phosphate ester (Alberts et al., 2002). As the final step of electron transport chain, high energy electrons are loaded (together with H^+) on to $NADP^+$, converting it to NADPH, and all of these reactions are confined to chloroplast (Alberts et al., 2002). In carbon fixation process, three molecules of ATP and two molecules of NADPH are consumed for each molecule of carbon dioxide (CO_2) converted in to carbohydrate. The net equation is: $3C02 + 9ATP + 6NADPH + water \rightarrow Glyceraldehyde\ 3 - phosphate + 8P_i + 9ADP + 6NADP^+$(Alberts et al., 2002). Orthophosphate in the chloroplast stroma serve as a substrate for ATP synthesis (Carstensen et al., 2018).

Recent work exhibited that P deficiency reduces the orthophosphate concentration (Pi) in the chloroplast stroma which may led to inhibit the process of ATP synthesis (Carstensen et al., 2018; Karlsson et al., 2015), and protons start accumulated in the thylakoids and cause lumen acidification, which stop linear electron flow. Inadequate

plastoquinol (PQH2) oxidation retards electron transport to the cytochrome *b6f complex*. However, the electron transfer rate of PSI is increased under steady state growth light conditions and is limited under high light conditions. P deficiency increased electron transport through PSI increases the levels of NADPH, whereas ATP production remains limited and reduced CO_2 assimilation. Changes in supply of Pi in the stroma reduced the ATP levels and consequently reduced, but no changes in the photosynthetic machinery composition were observed (Karlsson et al., 2015). P deficiency immediately affects CO2 fixation, but does not appear to stop it, as P deficient plants typically remains green and do not develop leaf chlorosis. Phosphorus starvation significantly reduced $NADP^+$ in to NADPH, indicating that a large fraction of $NADP^+$ remains in the reduced form NADPH, because it cannot be utilized in the Calvin cycle due to ATP limitation, and because of higher PS1 activity (Carstensen et al., 2018). NADPH concentration was significantly higher under P deficient treatment as compared to P supply treatments.

DM application improved photosynthesis and plant growth due to slow release of nutrients (Salehi et al., 2017). Additionally, DM application significantly increased photosynthesis rate as compared to barley mulch, poultry manure and mineral fertilizes (Efthimiadou et al., 2009). Our results has demonstrated that, manure with high and low P concentration significantly increased the photosynthesis rate of Yukon R as compared to control during 2016 (Table 2.11). However, G × P interaction was non-significant in 2017 and this might be due to variation in environmental condition i.e. less rain possibly reduced the P uptake and availability. Plant physiological processes, such as transpiration and photosynthesis response are dependent on the rapidity, severity, and duration of drought

stress (Zhang et al., 2012). Reduced soil moisture due to less rain closed the stomata to prevent desiccation (Yan et al., 2011) and consequently, affected photosynthesis rate due to decreased level of CO_2 at chloroplast level (Long et al., 2006).

2.5.1.4. Root shoot ratio

Root morphological traits i.e. root length, volume and density significantly increased under P deficiency, whereas sufficient P supply increased growth and total root biomass (Hill et al., 2006). Previous studies found that plants grown under sufficient P conditions led to more accumulation of P in shoots than roots and indicated that shoots are strong source of P for photosynthesis processes (Rao et al., 1989). Reduction in photosynthesis under P deficiency caused negative effects on leaf expansion cells which is the consequence of carbohydrates deficiency (Louw-gaume et al., 2010; Rao et al., 1989). Roots are larger sink than leaves to allocate carbohydrates (Louw-gaume et al., 2010; Rao et al., 1989). As a result of reduction in photosynthesis process under P deficiency, triose export from chloroplast would be reduced and starch accumulate in leaf tissue, while the export of carbohydrates to roots decreased (Rao et al., 1989). Roots are generally considered as larger sinks than leaves under P deficiency, since the root shoot ratio usually increased (Wissuwa et al., 2005). In this study, similar results were observed where the root to shoot ratio of silage corn genotypes were significantly higher; whereas photosynthesis decreased in the control led to export of P from shoots to root for adaptation to low P stress which may account for the increase root to shoot ratio. Our findings supported by that of earlier studies (Basirat et al., 2011; Li et al., 2009; Louw-gaume et al., 2010; Wissuwa et

al., 2005) suggest that P deficiency significantly increased root to shoot ratio of under P deficient conditions.

2.5.1.5. Plant height (PH)

Phosphorus plays an important role in plant growth rate by enhancing cell division (Kavanova et al., 2006) and cell elongation (Radin and Eidenbock, 1984) in several crops. Amujoyegbe et al., (2007) found that manure application significantly increased PH of corn through optimum supply of P which may lead to increased internode length and nodes formation of stems. In present studies, our (G × P) results indicated that Yukon R attained higher PH, when amended with high P manure, whereas, non-significant interaction was observed in 2017. This increment in PH occurred due to exclusive role of P in cell division and cell elongation. Additionally, P fertilizers application at early growth stages is known to be an effective strategy for stimulating corn root development and establishment of good root architecture and increasing yields (Shen et al., 2013). Efthimiadou et al., (2009) also found that manure application produced positive influence on PH of corn compared to inorganic fertilizers. Highest PH noted in Yukon R might be due to cultivar variability among the genotypes (Maryam et al., 2012). During 2017, decrease in PH of silage corn was observed compared to 2016, and this reduction in PH occurred due to less rainfall in month of July and August (Figure 2.3.1) which might hampered PH in silage corn in present study. Soil moisture deficit significantly affect the crop growth rate by decreasing the nutrients uptake and reduce LA, photosynthesis rate, cell division and elongation and finally PH in crops. Our results corroborate the findings of Maryam et al., (2012), who

reported that water stress significantly affect the process of cell division and elongation which can lead to decreased plant height. In another study conducted by Zhang et al., (2012) observed that soil moisture deficit affects photosynthesis rate, and consequently reduction of stem internodes length led to decreased plant height (Abrokwah et al., 2017).

2.5.1.6. Dry matter yield

Environment (temperature, rain, radiation, transpiration etc.), genotypes or plant species and management practices (fertilizers application, sowing dates, cultural practices, seeding methods, weed control etc.) are the major drivers of DMY (Martin et al., 2014). Basically, DMY is the outcome of interception of PAR by plant leaves and the ability of plants canopy architecture to transform the intercepted PAR into biomass production (Portes and Melo, 2014). Results of our study demonstrated that high P manure treatment increased LA, chlorophyll contents and photosynthesis rate which consequently led to increased DMY (Table 2.15). In previous studies conducted by Amujoyegbe et al., (2007) and Efthimiadou et al., (2009), authors reported that manure application significantly improved LA, chlorophyll contents, photosynthesis and dry matter yield of silage corn as compared to inorganic fertilizer application. Manure application improved agronomic performance of silage corn genotypes, because nutrients are slowly released from DM and available for long time in soil compared to inorganic fertilizer. Lowest DMY in control treatment could be attributed due to lower P availability that might have reduced LA, number of leaves, photosynthesis rate and finally DMY (Table 2.15). In another study conducted by Salehi et al., (2017) found that dry matter accumulation significantly

increased with manure application due to gradual release of nutrients which improved photosynthesis rate, plant growth and finally DMY.

In 2017, due to low rain and consequently low moisture availability caused water and nutrient stress at pre-anthesis and anthesis stages of silage corn, which significantly reduced DMY compared to 2016. Moreover, LA, chlorophyll contents, photosynthesis, shoot dry weight and plant height of silage corn decreased, possibly due to reduced nutrients uptake, which may have led to reduced DMY. Plants cope with drought conditions by closing stomata to prevent water loss which may lead to decreased photosynthesis rate and finally DMY of silage corn. Previous studies also reported that drought stress reduced LA, chlorophyll contents, plant height, photosynthesis and DMY in several crops (Abrokwah et al., 2017; Bouazzama et al., 2012; Maryam et al., 2012; Yan et al., 2011).

Pearson's correlation analysis showed positive and strong correlation between DMY of silage corn genotypes and LA, chlorophyll contents, photosynthesis rate, shoot dry weight and plant height (Table 2.27). Feng et al. (2016) also reported positive correlation between leaf area index, chlorophyll contents, photosynthesis rate and DMY of cotton. Results of present study conclude that high P manure application significantly improved agronomic performance and dry matter yield of silage corn in western Newfoundland.

2.5.2. Biochemical attributes

2.5.2.1. Soil rhizosphere pH

Soil rhizosphere pH is an important factor that modify physiochemical properties and microbial community composition (Gregory and Hinsinger, 1999), and determine the availability of macro and micro nutrients (Hinsinger, 2001). Variation in soil rhizosphere pH under different nutrient regimes might be due to imbalance uptake of cations and anions by genotypes or species (Hinsinger, 2001; Li et al., 2008). Basically, DM or organic fertilizers have high pH and their application enhance soil pH and SOC than synthetic fertilizers (Dong et al., 2012; Lapa et al., 2011). In our study, the rhizosphere soil pH was slightly higher in manure treatments than control (Table 2.18), which suggest that manure either with low or high P application could alleviate soil acidification by increasing soil organic matter (SOM), improving the soil structure, and enhancing the soil base saturation percentage (Dong et al., 2012; Li et al., 2010; Zhang et al., 2009). Application of inorganic fertilizers (alkaline in nature) would return some alkaline substance to soils which may led to increase the soil pH (Dong et al., 2012). Manure application significantly increased soil pH than no manure applied soils which was attributed to buffering from bicarbonates anion and organic acids (Whalen et al., 2000). Manure decomposition process also increase the soil pH due to the decarboxylation reaction in which protons (H^+) are consumed and CO_2 is released: $R-CO-COO^- + H^+ \rightarrow R-CHO + CO_2$ (Yan et al., 1996). Li et al., (2008) found that soil pH varied among rice genotypes and this difference occurred due to complex rhizosphere processes. Organic fertilizer sources or manure are able to stabilize

and increase the soil pH (Dong et al., 2012) and we found that DM application stabilize the pH during first year and significantly increased in 2017 (Table 2.18)

2.5.2.2. Acid phosphatase activity

Soil enzymes are instrumental in organic matter decomposition and nutrients turnover. Therefore, soil enzymes activities are indicators of soil health and early indicators of land use changes caused by agricultural practices (Paz-Ferreiro et al., 2007). Plants and soil microorganisms have developed several mechanisms to mobilize organic P and solubilize bound inorganic P through exudation of protons and organic ligands such as oxalate and citrate (Hinsinger, 2001). Extracellular acid phosphatase and other enzymes activities fluctuate rapidly due to fertilizers, complex rhizosphere processes, biological properties, soil physio-chemical properties and environmental conditions (Paz-Ferreiro et al., 2011). Manure contains organic P which is mineralized by phosphatases in to inorganic P before plants uptake; soil microbial and enzyme activities significantly affect the P hydrolysis (Waldrip et al., 2012). Organic P mineralization is controlled by three enzymes such as acid phosphatase, alkaline phosphatase and phosphodiesterase (Waldrip et al., 2012). Both acid and alkaline phosphatase hydrolyze relatively nonspecific and a range of low molecular-weight P compounds, including mononucleotides, phytate, sugar phosphates, and polyphosphates, whereas phosphodiesterase catalyzes the hydrolysis of nucleic acids and phospholipids (Turner et al., 2002). Mineral P fertilizers application significantly decreased the acid phosphatase activity, because plants and microorganisms easily draw the P from readily available sources and do not have to mineralize organic P

(Spohn and Kuzyakov, 2013). High P manure significantly increased Ap-ase activities compared to inorganic P source in the present studies (Table 2.19), which might suggest that manure application may stimulate Ap-ase activity by providing microorganisms with organic sources of C, N and P (Li et al., 2016; Zhang et al., 2015). Silage corn genotypes also exhibited significant difference in rhizosphere Ap-ase activity, which might be due to difference in genotypes potential of roots respond to various P fertilizer sources which affect the Ap-ase activity in rhizosphere. Root exudates (protons, oxalate and citrate) potential varied among the silage corn genotypes which affect the solubilization of C, N and P, and shape the microbial community to decompose the manure by secretion of various enzymes. We conclude that high or low P manure application significantly increased Ap-ase activity to mineralize the organic P by increasing soil microbial communities. Our results are in agreement with studies reported by earlier researchers (Colvan et al., 2001; Leytem et al., 2011; Waldrip et al., 2012).

2.5.3. Soil PLFA profiles

Microbial community composition in the soil rhizosphere can be altered due to physio-chemical properties of soil, chemical nature of root exudates, fertilizer sources and their management practices, and plant growth stage (Li et al., 2014b, 2014a; Wang et al., 2017). Soil microbial communities are sensitive to external application of N and P, and decreased in response to nitrogen application (Liu et al., 2013; Pan et al., 2014). P fertilization significantly increased diversity and abundance of microbial community in tropical forest, grasslands and pastures (Liu et al., 2013; Pan et al., 2014), specifically G^-

bacteria, arbuscular mycorrhizal fungi (AMF), ΣBPLFAs and ΣPLFAs compared to control, whereas mean abundance of G^+ bacteria did not differ among treatments (Liu et al., 2013). Apparently, P fertilization in tropical forest enhanced SOM transformation and increased C availability which may led to increased soil microbial biomass (Liu et al., 2013).

Phospholipid fatty acids (PLFAs) are major component of membranes in all living cells, and several studies have documented that fertilizer management practices have altered the abundance and composition of microbial community in paddy and maize (Guo and Wang, 2009; Wei et al., 2017; Zhang et al., 2015). Significant differences were observed in microbial communities of two maize genotypes due to secretion of root exudates such as sugars, amino acids, organic acids and hormones which enhanced greater bacterial growth in soil rhizosphere and higher availability of C (Wang et al., 2017). Agricultural soils are generally C limited and application of manure stimulate the growth of various microbial groups by increasing SOC labile fractions, N and P pool in soil, which probably could serve as major energy sources for microorganisms (Demoling et al., 2007). In the present study, High and low P manure application might have provided readily available substrates for the microbial community, whereas small increase of labile organic C under inorganic fertilization might be not enough to support the substantial growth of microorganisms (Li et al., 2018). Diversity in soil microbial community and abundance was significantly increased due to P rich environment resulted from long term P fertilization research trial, in comparison to control (Tan et al., 2013). Bacterial community were most sensitive to fertilizers and environmental conditions in soil, as they have a much short

turnover time than fungi (Lazcano et al., 2013). Inorganic P fertilization not only influenced the soil microbial abundances but also altered bacterial composition (Liu et al., 2013).

Wei et al. (2017) reported that G^- bacteria proliferate and grows faster soon after the addition of organic materials and then decrease and also facilitate the growth of other slow growing microorganisms such as G+ bacteria or fungi. It has been observed in a previous study that DM application significantly increased G^- bacterial biomass relative to G^+ bacterial biomass due to higher availability of soluble carbon over a long part of the year than inorganic fertilization (Peacock et al., 2001). Moreover, higher proportion of G^- bacteria usually occurs following a shift from nutrient deficient (oligotrophic) condition to nutrient rich (copiotrophic) conditions, and this pattern was observed in soil amended with P fertilizers (Tan et al., 2013). In present study, we found that G^+ and G^- bacteria benefited from manure application, and there was a significant increase irrespective of low or high P manure treatments compared to inorganic P and control. However, G^- bacterial PLFAs was predominantly higher in manure with high P source compared to inorganic P treatment in both years (Table 2.24). Our results are in line with that of Wei et al. (2017), where changes in G^+ and G^- bacterial community were strongly related to increase in soil organic C added by application of manure. In general, a lower G^+/G^- ratio is an indicator of better soil nutrition (Rajendran et al., 1997). In present studies, we found that G^- bacteria significantly increased relative to G^+ irrespective of low or high P source used (Table 2.24). This suggests that G^- bacteria was sensitive to oligotrophic conditions (Tan et al., 2013), and often stimulated by adding organic matter resulting in lower G^+/G^- bacteria ratio. In another

study conducted by Zhang et al., (2015) supported that same argument that G^+/G^- bacteria ratio was significantly reduced in organic matter amended treatments.

Fungi play an important role in C and nutrient cycling in agricultural ecosystems and are sensitive to fertilizers application (Li et al., 2018). In general, lower fungal population relative to bacteria is common in agricultural ecosystems due to intensive physical disturbance (tillage) and changes in amount, type and source of fertilizers inputs as compared to undisturbed soils (zero tillage) (Mbuthia et al., 2015). Inorganic P application significantly increased bacterial PLFAs and relative abundance of fungi, which obtain C from their host plants (Liu et al., 2013). Our results showed that high P manure application significantly enhanced the active fungal biomass in both years compared to control and inorganic P source that might be due to additional C attained by manure application and served as major source of energy for active fungi whereas, small increase of labile organic C under inorganic fertilization and control treatments reduced growth (Liu et al., 2013). Another possible reason of higher active fungi biomass in the high P manure application in our study could be due to DM increased the soil rhizosphere pH of silage corn genotypes, as this is often associated with increased fungal population in soil (Rousk et al., 2010). Higher F/B ratio reflect the relative abundance of microbial population which is an important indicator of a strong soil ecosystem buffering capacity and more sustainable land use (De Vries et al., 2006). In current study, inconsistency in F/B ratio among P sources during 2016 was noted, compared to 2017, where manure application enhanced F/B ratio compared to inorganic P and control (Table 2.24). This anomaly in F/B ratio might be due to higher organic matter contents (3.01%) in soil and furthermore, manure applied

in soil contains higher percentage of P, N and C in high P manure treatment during 2017 relative to 2016 organic matter contents (2.98%) (Table 2.1 & 2.5), and fungi might have played a larger role in organic matter decomposition and C cycling (Buyer et al., 2010; De Vries et al., 2006; Liu et al., 2013). Earlier studies also reported that inorganic fertilizers reduced F/B ratio, while manure application increased fungi growth and thus increased F/B ratios (Buyer et al., 2010).

Different plant species and genotypes release secondary metabolites (organic acids, sugars, amino acids, lipids, enzymes and aromatic compounds) in their rhizosphere, which in turn stimulate the growth of dormant microbial species (Li et al., 2014b; Szoboszlay et al., 2015). In the present studies, we found that silage corn genotypes significantly altered microbial community composition in their rhizosphere, particularly in Yukon R root rhizosphere (Table 2.23), Yukon R presumably released more secondary metabolites or root exudates which in turn altered microbial composition and abundance. High P manure significantly increased G^- bacteria, fungi, eukaryotes, ΣBPLFAs and ΣPLFAs (Table 2.24), that might be associated with higher organic C and extracellular enzyme activities involved in C, N and P mineralization compared to inorganic P and control (Table 2.19), indicating that P availability could be a limiting factor for soil bacterial growth (Liu et al., 2013; Wei et al., 2017).

Our PCA results supports the finding that field scale changes in microbial community structure were observed based on the dairy manure applied and induced changes in soil biochemical attributes (pH, Ap-ase and SAP). Phosphorus addition in paddy

soil influenced both bacterial and fungal communities as compared to control (Guo and Wang, 2009). Previous studies found that SOC, N, P and pH are the main drivers of soil microbial community composition (Liu et al., 2013; Wei et al., 2017; Zhang et al., 2015). However, in our study we found that soil pH, Ap-ase and SAP under different P sources were all important factors in shaping the active soil microbial community (Figure 2.4.1b & Figure 2.4.2b). This is consistent with results of (Liu et al., 2013; Wei et al., 2017), who reported that soil pH, SOC and nutrients availability were the key determinants that affected the soil microbial community.

Pearson's correlation analysis showed that Ap-ase and SAP were significantly and positively correlated with active fungal biomass, ΣBPLFAs biomass and ΣPLFAs in 2016. However, Ap-ase activity was not significantly correlated with ΣBPLFAs biomass and ΣPLFAs during 2017 (Table 2.28). Our results corroborate the findings of previous findings demonstrating that SAP and Ap-ase were strongly correlated with microbial communities (Wei et al., 2017; Zhang et al., 2015). In the present study, we found that organic and inorganic P sources had significant effects on agronomic performance, biochemical attributes and soil microbial community composition in silage corn. High P manure application increased overall active microbial composition and abundance which might have contributed to efficient cycling of N, P along with improved overall agronomic performance of silage corn and soil biochemical attributes and microbial community composition.

2.6. Conclusion

In two years field trial, we found that organic and inorganic P sources significantly influenced the agronomic performance, soil biochemical attributes, and microbial community composition and abundance of silage corn genotypes in podzolic soils of western Newfoundland. Results suggest that DM with high P (contains high N and SOC) significantly improved the LA, chlorophyll contents, photosynthesis rate, PH and DMY. SAP, Ap-ase, and active microbial community composition and abundance were also improved with high P manure compared to control. Yukon R and DKC 26-28RIB showed superior agronomic performance and produced higher DMY compared to other genotypes, suggesting that these two genotypes has the potential to be successfully cultivated in podzolic soils under cool climatic conditions of western NL. High P manure source also showed a positive and strong relationship with agronomic performance parameters and DMY, biochemical attributes and active soil microbial community, indicating that high P manure application could be a useful practice and strategy for producing higher DMY of silage corn in podzolic soils. The addition of DM increased bacterial PLFAs and soil P cycling and ecological buffering capacity, which resulted in higher proportion of G- and fungal population relative to the control. PCA analysis also supported the argument that Yukon R and DKC26-28RIB genotypes showed superior agronomic performance and produced higher biomass. Taking all together, we conclude that high P dairy manure application could be a rational strategy to sustain crop growth, dry matter yield, and soil health compared to inorganic P source. Further studies are required to understand how root

exudates affects soil microbial community composition and diversity, P cycling and related key soil functions in podzolic soils, to sustain biomass yield of silage corn.

2.7. References

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Chapter 3

3. Evaluating the forage production and quality of silage corn under organic and inorganic phosphorus sources

3.1. Abstract

The dairy industry is a major contributor to the food security and economy of Newfoundland and Labrador (NL). However, dairy sector faces acute shortage of quality forage production to meet the needs of dairy farmers. To address this challenge, a field experiment was conducted at Pynn's Brook research station, Pasadena, to determine the forage production and quality of five silage corn genotypes following dairy manure and inorganic phosphorus applications. Experimental treatments were: dairy manure with high phosphorous (DM_1), dairy manure with low P (DM_2), inorganic P (IP) and control (no P applications) and five silage corn genotypes (Fusion-RR, Yukon-R, A4177G3-RIB, DKC23-17RIB, DKC26-28RIB). Results revealed that organic P source (DM1) had significant effects on forage production and NFC compared to control but non-significant effects on forage fiber contents (ADF, NDF) and energy parameters (NEM, NEG, NEL). However, DM1 was statistically at par with DM2 and IP treatments while comparing NFC contents. Significant genotypic difference in forage production and quality parameters were observed. For instance, DKC26-28RIB, and Yukon-R produced higher forage production, however, A4177G3–RIB genotype produced superior quality forage. For instance, P, K, Mg, CP, AP, TDN, NEM and NEG was higher in A4177G3–RIB compared to other genotypes.

3.2. Introduction

Sustainable food production and security is the prime objective of agricultural production systems (Tilman et al. 2011). The global population is predicted to grow to 9.2 billion through 2050, necessitating enormous increases in food production, as well as, reductions in waste (Fedoroff 2015). It is estimated that annual cereal and meat production will need to rise to about 900 and 200 million tonnes respectively to feed the world (FAO 2009). To achieve this, more animal feed and raw materials will be needed to increase milk and meat production to meet the needs of the ever increasing world population (Ittersum 2011). Enhancing milk production and quality in response to the increasing global demands for dairy products requires more sustainable, productive, economically viable, and environmentally beneficial forage cropping systems (Martin et al. 2017). Historically, dairy farmers grow forages, pasture, grasses and purchase minimal protein, high energy feed stock, minerals, and vitamins to meet cattle nutrient requirements (Tauer and Mishra 2006; USDA-NASS 1989; 2014; Write 2009).

Corn (*Zea mays* L.) is one of the most important food and forage crops contributing to the global food security for human and animals (Ranum et al. 2014). Silage corn is a high-energy crop with multifaceted benefits compared to other forage species (Khan et al. 2012; Khan et al. 2015; Phipps et al. 2010). Dairy farmers primarily grow corn for silage and grain in addition to alfalfa, soybeans, oats, wheat, barley, and sorghum (Gallo et al. 2013). Interestingly, acreage under hay crops, grasses, and green chop has declined in the last two decades, whereas silage corn production is increased by 33%, and alfalfa hay

production decreased by 75% (USDA-NASS 2014). Increased silage corn production mirrors the growing reliance on silage corn, as a primary forage source for livestock and dairy sector (Khan et al. 2015; Robinson 2008). This increasing trend has allowed dairy farmers to increase herd size, and stocking rates to approximately 27% more cows per unit land area when silage corn replaces alfalfa silage in the cropping systems (Powell et al. 2016). Silage corn is a preferred forage compared to alfalfa and other forages due to higher biomass production potential, energy content, more uniform quality and one cut forage harvest (Ericsson and Nilsson 2006; Kirkland et al. 2005; Phipps et al. 2016; Rankin 2014). Additionally, it is highly palatable, digestible and easy to ensile due to high soluble sugar contents (Karsten et al. 2003; Kwabiah et al. 2003). Owing to high energy contents of silage corn, milk production was significantly increased compared to other grasses as a sole source of energy (Fitzgerald and Murphy 1999). Consequently, dairy farmers need sustainable supply of quality forage production to meet forage security that could be achieved through adaptations of good agricultural practices that make use of local resources to enhance forage production, quality and diversify the forage production systems.

Dairy manure (DM) is historically known as a rich source of plant nutrients, and good soil amendment for plant growth and higher biomass production (Hart et al. 1997; Jarvis 1993; Newton et al. 2003a; Pain et al. 1989; Walsh et al. 2012). For instance, a single dairy cow produces about 10 to 30 kg of phosphorus (P), and 90 to 150 kg of total nitrogen each year (ASAE 2005), in addition to other macro and micro nutrients (Eghball et al. 2002; Kurt and Torsten 2012; Warman and Cooper 2000). Therefore, DM application could be a promising and sustainable P resource to enhance forage production and on-farm nutrient

recycling (Agriculture 2002; Leytem et al. 2014; Seleiman et al. 2012; Seleiman et al. 2017). Phosphorus is one of the essential mineral nutrients required for plant growth and development (Glass et al. 1980; Richardson et al. 2009; Schachtman et al. 1998); however, at the same time it is one of the most immobile and inaccessible nutrients present in soils (Holford 1997; Lynch 2011). Therefore, P deficiency in crop plants may lead to stunted growth and reduced crop yields (Assuero et al. 2004; Barry and Miller 1989; Plénet et al. 2000a; Plénet et al. 2000b; Plénet et al. 2000c). Depending on the soil pH, plant roots take up P either in the form of primary orthophosphate ($H_2PO_4^-$) or secondary orthophosphate (HPO_4^{2-}) anions. However, $H_2PO_4^-$ is the predominant form in acidic soils (Kulhánek et al. 2007; Raghothama 1999b). The root morphology and architecture of crop plants play an important role in exploring the rhizosphere area, which are associated with soil rhizosphere microorganisms (actinomycetes, protozoans, bacteria, and fungi), release organic compounds (phosphatases and organic acids) and inorganic P in the rhizosphere (Fageria et al. 2014; Kulhánek et al. 2007; Lynch 2011; Raghothama 1999a; Rausch and Bucher 2002).

Generally, P fertilization cause rapid immobilization in soils due to its higher reaction capacities with organic matter or cations (Ca, K, Al, Fe) in alkaline and acidic soils causing unavailability of P for the plant uptake (Hinsinger 2001; Lynch 2011; Vance et al. 2003). Due to such higher reactivity in acidic and alkaline/calcareous soils, approximately 70 percent of global croplands have become P deficient making inorganic P fertilization an inevitable input in intensive agriculture. Furthermore, depleting rock phosphate resources in the next 50-100 years is also a growing concern for the agriculture production systems

(Cordell et al. 2009; Vance et al. 2003). Hence, using DM as a plant nutrient source can be a suitable alternative option for sustainable food and forage production, particularly in territories of expanding dairy industry (Newton et al. 2003a). This will further enhance the efficient utilization of abundantly available DM as a P source, while simultaneously reduce the dependency on non-renewable inorganic phosphate rocks source (Cordell et al. 2009; Vance and Chiou 2011; Vance et al. 2003). It has been reported that DM application enhanced on-farm nutrient use efficiency in silage corn compared to other forage grasses (Ketterings et al. 2007; Newton et al. 2003b; Roth and Heinrichs 2001). Moreover, DM application can enhance mineral uptake, milk and forage production (Cherney et al., 2015). Higher protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), and total digestible nutrients (TDN) were also noted in forage obtained from silage corn following DM application compared to inorganic nutrient sources (Moreno-Reséndez et al. 2017).

Earlier researchers reported variations in P use efficiency in different crop genotypes, most probably due to their genetic makeup (Balemi and Schenk 2009a;b; Corrales et al. 2007; Ozturk et al. 2005). Similarly, different silage corn hybrids expressed variations in forage proteins, minerals, ADF, NDF, TDN and forage energies (Amodu et al. 2014; Faisal et al. 2013; Kwabiah 2005; Peña-Ramos et al. 2002; Schwab et al. 2003). Furthermore, different P application rates also displayed significant effects on biomass production and nutritional aspects of different forage species including corn (Amin 2011; Ayub et al. 1999; Eghball and Power 1999; Hazary et al. 2015; Hirzel et al. 2007a; Lentz and Ippolito 2012; Malhi et al. 1992; Mazza et al. 2012; Polat et al. 2007; Roy and Khandaker 2010; Toth et al. 2006); however, information given above does not provide

substantial knowledge to elucidate the effects of organic (DM with high and low P) and inorganic fertilizer P source on forage quality of silage corn genotypes. It is also important to assess the adaptability of these corn genotypes for optimum plant growth, and forage quality in cool climate production systems (low crop heating unit receiving areas). Therefore, we hypothesized that organic (DM) and inorganic fertilizer P source will improve forage minerals, protein, fiber contents and forage energies of silage corn genotypes in low corn heat unit (CHU) receiving areas. Hence, a field research trial was conducted for three years to investigate the effects of organic (DM) and inorganic P fertilizer source on forage quality of silage corn genotypes.

3.3. Material and methods

3.3.1. Experimental location and treatments

A field research trial was carried out at Pynn's Brook Research Station, Pasadena (49° 04' 20" N, 57° 33' 35" W), Newfoundland and Labrador (NL) for three years (2015 - 2017). The soil, reddish brown to brown, has developed on gravelly sandy fluvial deposit of mixed lithology, with >100 cm depth to bedrock, and soil samples for the study site revealed a gravelly loamy sand soil [sand = 82.0% (±3.4); silt = 11.6% (±2.4); clay = 6.4% (±1.2], which is classified as orthic Humo-ferric podzol (Kirby 1988). Five Roundup® ready silage corn genotypes were selected based on their CHU requirements to assess the forage production potential and forage nutritional quality in cool climate production systems. The genotypes were obtained from three sources; Brett Young (Fusion-RR with 2200 CHU; Yukon-R with 2150 CHU), Pride Seeds (A4177G3-RIB with 2175 CHU), and

DEKALB Canada (DKC23-17RIB with 2075 CHU and DKC26-28RIB with 2150 CHU). The four P sources used in this experiment were: two dairy manure (DM) sources collected from two dairy farms with either low or high P concentrations, triple superphosphate (0-45-0) as inorganic P (IP) and control (no P applications) (Table 3.1). To assess the P concentration of the DM treatments, well-agitated DM samples were collected from western NL dairy farms and were sent to the Soil, Plant and Feed Laboratory, Department of Fisheries and Land resources, St. John's, NL. DM sample with high and low P concentration were designated as DM_1 and DM_2, respectively (Table 3.1). During each growing season, well-agitated DM samples were collected from the same two selected dairy farms and were sent to the laboratory for complete nutrient analyses prior to field application. DM was applied to the respective treatment plots @ 30,000 L ha^{-1} according to the local dairy farmers practice and was thoroughly incorporated in the top 15-20 cm soil layer before crop seeding. The remaining crop nutrient (NPK) requirements were supplemented through inorganic fertilizers in all treatments except P in control P treatment based on the soil and manure analyses reports (Table 3.1 & Table 3.2), and regional recommendations for silage corn production. Briefly DM (DM_1 or DM_2) treated plots received P from dairy manure plus from inorganic P fertilizer to meet the crop P requirements. It is pertinent to mention here that location of experimental plots was not changed during all three growing seasons to minimize error within treatments.

Table 3.1 Composition of phosphorus sources used in the current research trial under cool climatic conditions.

Treatments	Nutrients	2015	2016	2017
Control P (DM0)		No P applications		
Manure with high nutrients (DM1) @ 30,000 L ha^{-1} + remaining P requirements from inorganic fertilizer	pH	7.0	6.8	6.8
	Dry matter (%)	9.6	9.33	10.9
	Total nitrogen (%)	0.328	0.37	0.44
	Total phosphorus (%)	0.057	0.065	0.087
	Total potassium (%)	0.328	0.379	0.37
	Total calcium (%)	0.139	0.164	0.19
	Total magnesium (%)	0.065	0.069	0.077
Manure with low nutrients (DM2) @ 30,000 L ha^{-1}+ remaining P requirements from inorganic fertilizer	pH	7.3	7.0	7.1
	Dry matter (%)	3.5	3.57	1.7
	Total nitrogen (%)	0.148	0.140	0.12
	Total phosphorus (%)	0.031	0.028	0.017
	Total potassium (%)	0.134	0.119	0.12
	Total calcium (%)	0.074	0.059	0.042
	Total magnesium (%)	0.032	0.026	0.018
Inorganic P (IP) @ 110 kg ha^{-1}	Triple superphosphate			

Table 3.2 Soil physiochemical properties of experimental site before seeding

Soil parameters	2015	2016	2017
pH	6.8	6.4	6.8
Organic matter (%)	2.78	2.98	3.01
Phosphorus (mg L^{-1})	80	81	74
Potassium (mg L^{-1})	45	38	49
Calcium (mg L^{-1})	1643	1256	1120
Magnesium (mg L^{-1})	241	265	218

3.3.2. Crop husbandry

Silage corn genotypes were seeded with SAMCO system (SAMCO 2200 Agricultural Manufacturing, Limerick, Ireland) capable of seeding two corn rows, and simultaneously cover the seeded rows with plastic sheets. Biodegradable and partially perforated plastic sheets provide cover at 1m width for two adjacent rows to accumulate CHU during germination and early crop establishment. Plastic sheet provides sufficient CHU and is beneficial in cool climatic production systems (Kwabiah 2005), as the minimum temperature was lower than corn base temperature (10 °C) during germination and seedling establishment phase. Seeding was done @ 90900 plants per hectare on June 4, May 25th and May 23rd, and harvested on October 13, 18, and 13 during 2015, 2016 and 2017 growing seasons, respectively. Glyphosate (WeatherMax) herbicide was applied according to the instructions on the label (Monsanto Canada Inc.). One square meter (1 m^2) area from the centre of each plot was hand-cut at ground level at the black layer stage (R6), which is considered as the best crop harvesting stage for optimum forage nutritional quality (Bal et al. 1997; Wiersma et al. 1993). The fresh forage biomass was recorded per square meter, and a subsample was chopped into small pieces with knife and dried in a forced air oven (Shell Labs USA) at 65 °C until constant dry weight was attained. The dry matter content (g kg^{-1}) was then calculated based on the total fresh and dry weights of the sample as described by Kwabiah (2005). Total forage production was converted from 1 m^2 to per hectare on a dry matter basis (equations below), and was reported as Mg ha^{-1}.

$$\%\ \text{dry matter} = \frac{\text{Weight after drying}}{\text{Weight before drying}} \times 100$$

$$\text{Dry matter yields} = \text{Fresh biomass production} \times \%\ \text{dry matter}$$

3.3.3. Forage quality analysis

Three plants from each experimental plot were selected randomly for forage quality analysis. Whole plants were chopped, pooled and dried at 65 °C in a forced air oven (Shell Labs USA) until constant weight was obtained. The plant samples were then crushed to a powder form using a grinder mill (Wily Mill Standard Model-3, Arthur H. Thomas Co. Philadelphia, USA), and sieved through a 500 μM screen. Thereafter, sieved samples were sent to the Actlabs, (member laboratory of Dairy One Feed and Forage Analyses, Ithaca, New York USA) Ancaster, Ontario (ON) for forage quality analyses. Near infrared reflectance analysis (NIR) technique (Foss NIR System Model 6500 Win ISI II v1.5) was employed to determine forage minerals (phosphorus: P, potassium: K, calcium: Ca, magnesium: Mg), protein (crude protein: CP, available protein: AP), simple sugars (SS), fibers (acid detergent fiber: ADF, neutral detergent fiber: NDF) and non-fibrous carbohydrates (NFC). Total digestible nutrients (TDN) were assessed using summative equation based on forage quality components at maintenance level 1x (Weiss et al. 1992). Silage corn forage energy contents including net energy for lactation (NEL), net energy for maintenance (NEM), and net energy for gain (NEG) were calculated using National Research Council standard equations (2001) and Van Soest (Van Soest 1982) variable discount approach.

3.3.4. Statistical Analysis

The experiment was laid out in a factorial combination of five silage corn genotypes, four P sources, and managed in a randomized complete block design with three replications. The individual experimental plot was 4.8 × 1.5 meters with two rows orientated in east-west directions. The experimental treatments were maintained continuously for three years in the same plots to minimize error within treatments. Data were pooled and principal component analysis (PCA) was performed to determine the similarities or groupings of the genotypes, and P sources based on forage production and forage quality parameters. PCA analysis was conducted using XLStat (XLStat Premium 2017, Version 19.5). Based on the quadrants, one-way or two way ANOVA was performed to evaluate observed variables in each quadrant by employing the Statistix-10 software package (Analytical Software, FL, USA). Where treatment effects were significant, the means were compared with Fisher's least significant difference (LSD) test at $\alpha = 0.05$. Figures were prepared using XLStat (XLStat Premium 2017, Version 19.5) and Sigma Plot 13.0 (Systat Software Inc., San Jose, CA) software packages.

3.4. Results

3.4.1. Mineral composition, net energy for maintenance and for gain in silage corn genotypes (Q1)

Principal component analysis showed the first (F_1) and the second components (F_2) explained 36.28% and 25.34% of the total variance in the data set, respectively

(Figure 3.4.1a & b). Significant differences ($p<0.01$) in forage minerals (P, K, Mg), net energy for maintenance (NEM), net energy for gain (NEG) and total digestible nutrients (TDN) among five silage corn genotypes were observed (Figure 3.4.2). One–way ANOVA showed that A4177G3–RIB genotype showed higher forage minerals (P and Mg), energy (NEM and NEG) and TDN compared to other genotypes (Figure 3.4.2a-e). PCA also showed association of these qualitative traits with A4177G3–RIB and therefore, clustered in the same quadrant (Q1) (Figure 3.4.1b). If we compare the mineral composition among five genotypes, then high P (2.75 g kg^{-1}), and Mg (1.71 g kg^{-1}) was observed in A4177G3–RIB and Fusion–RR produced low minerals. Although, DKC26–28RIB was statistically at par with A4177G3–RIB and produced similar P. Significantly higher NEM (1.40 Mcal kg^{-1}) and NEG (0.82 Mcal kg^{-1}) were also produced by A4177G3–RIB, compared to low NEM and NEG were observed in DKC23–17RIB genotype. However, Fusion–RR and Yukon–R were statistically at par with A4177G3–RIB genotype and produced similar NEM and NEG (Figure 3.4.2 c & d). Higher TDN (639.17 g kg^{-1}) was also produced by A4177G3–RIB genotype, although statistically non-significant from Fusion–RR and Yukon–R (Figure 3.4.2e). Overall, A4177G3–RIB genotype was superior in producing minerals, energy contents and total digestible nutrient compared to other genotypes (Figure 3.4.2a–e).

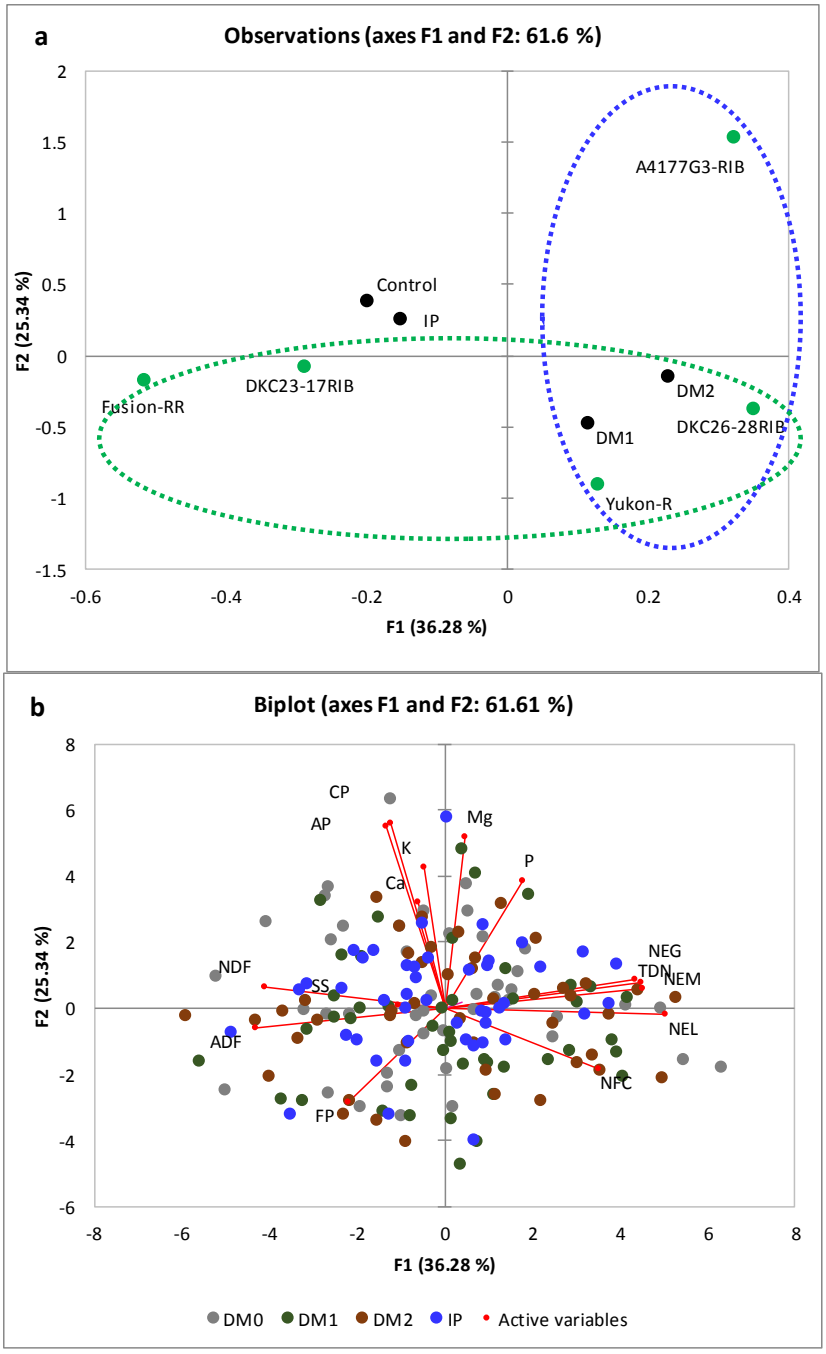

Figure 3.4.1 Principal component analysis of the first two components performed on the forage production and forage nutritional quality of five-silage corn genotypes affected by four phosphorus sources. **(a)** Observation plot showing segregation of silage corn genotypes and phosphorus sources based on the centroids on the F1 and F2 axis; and **(b)** Biplot showing relationship between different observations, forage production and nutritional quality in five silage corn genotypes treated with four P sources.

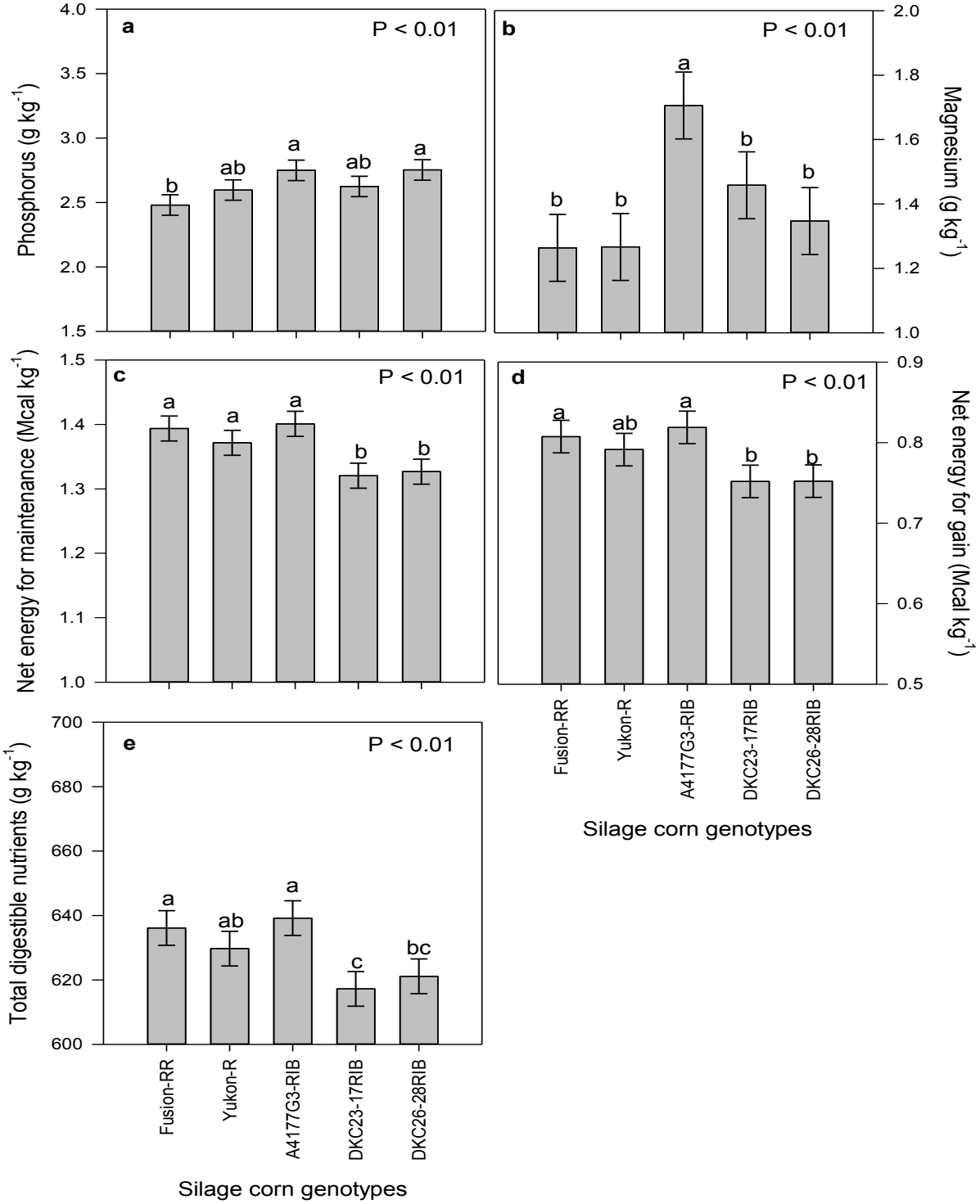

Figure 3.4.2 Quadrant 1 representing the quality matrix of silage corn genotypes (a)phosphorus, (b) magnesium, (c) net energy for maintenance, (d) net energy for gain, (e) total digestible nutrients

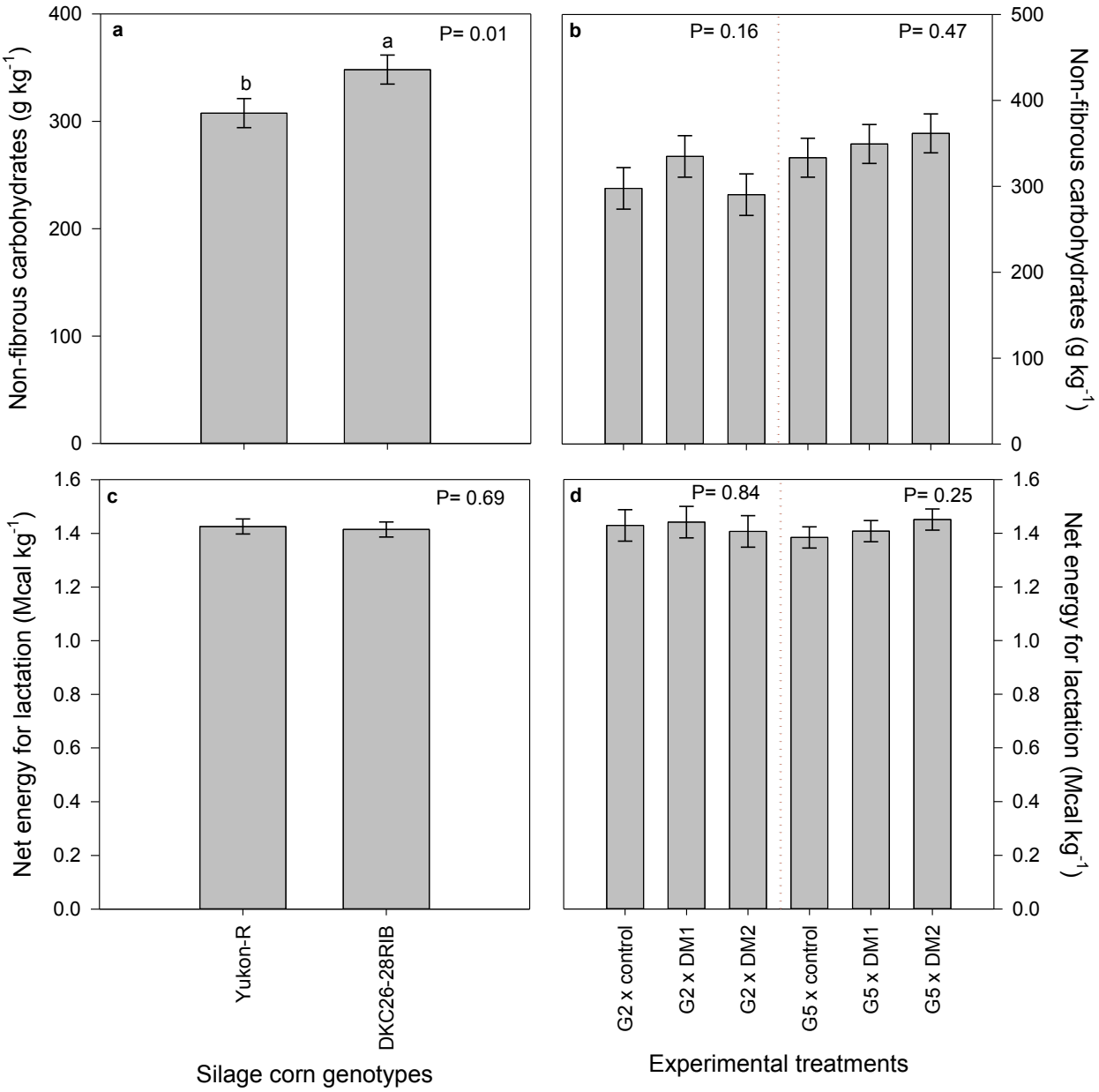

Figure 3.4.3 Quadrant 2 representing the quality matrix of silage corn genotypes (a) non-fibrous carbohydrates, and (c) net energy for lactation; as well as, the effect of silage corn genotypes and phosphorus sources on (b) non-fibrous carbohydrates, and (d) net energy for lactation.

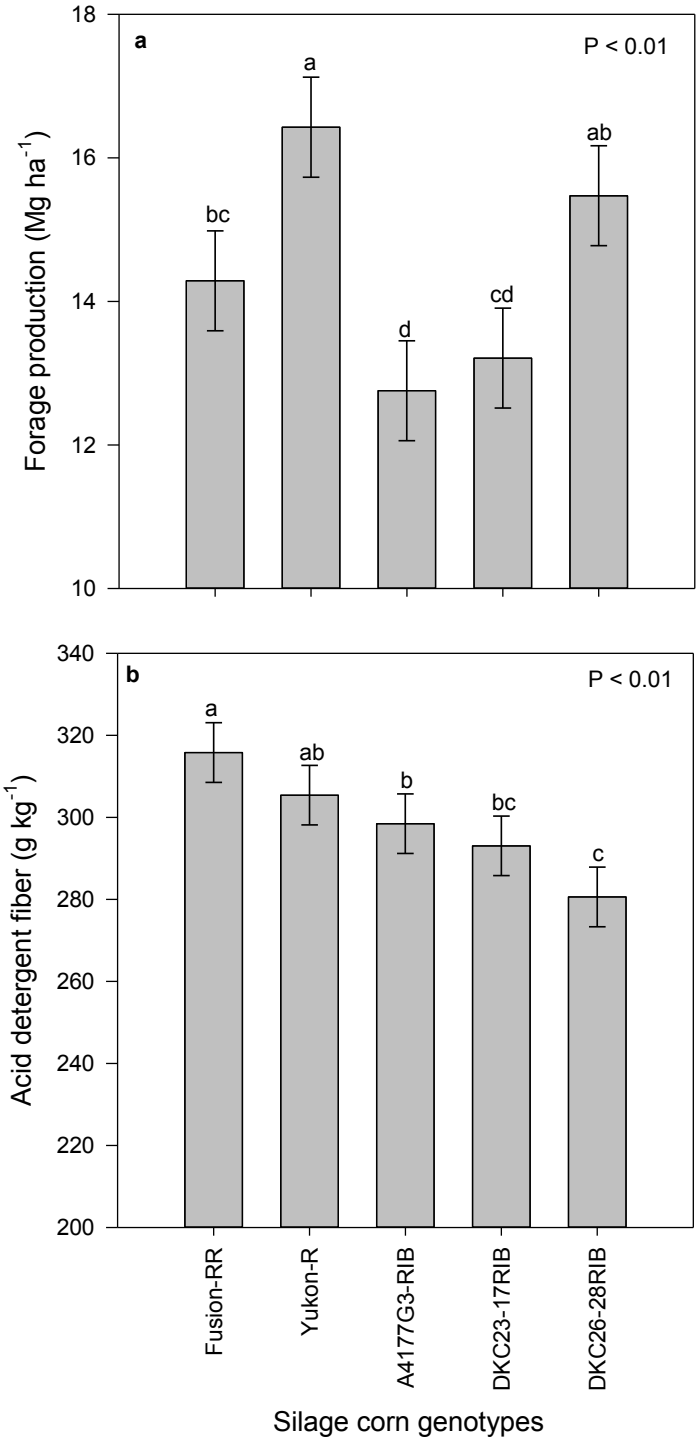

Figure 3.4.4 The quadrant 3 represnts (a) forage production potentials and (b) acid detergent fiber.

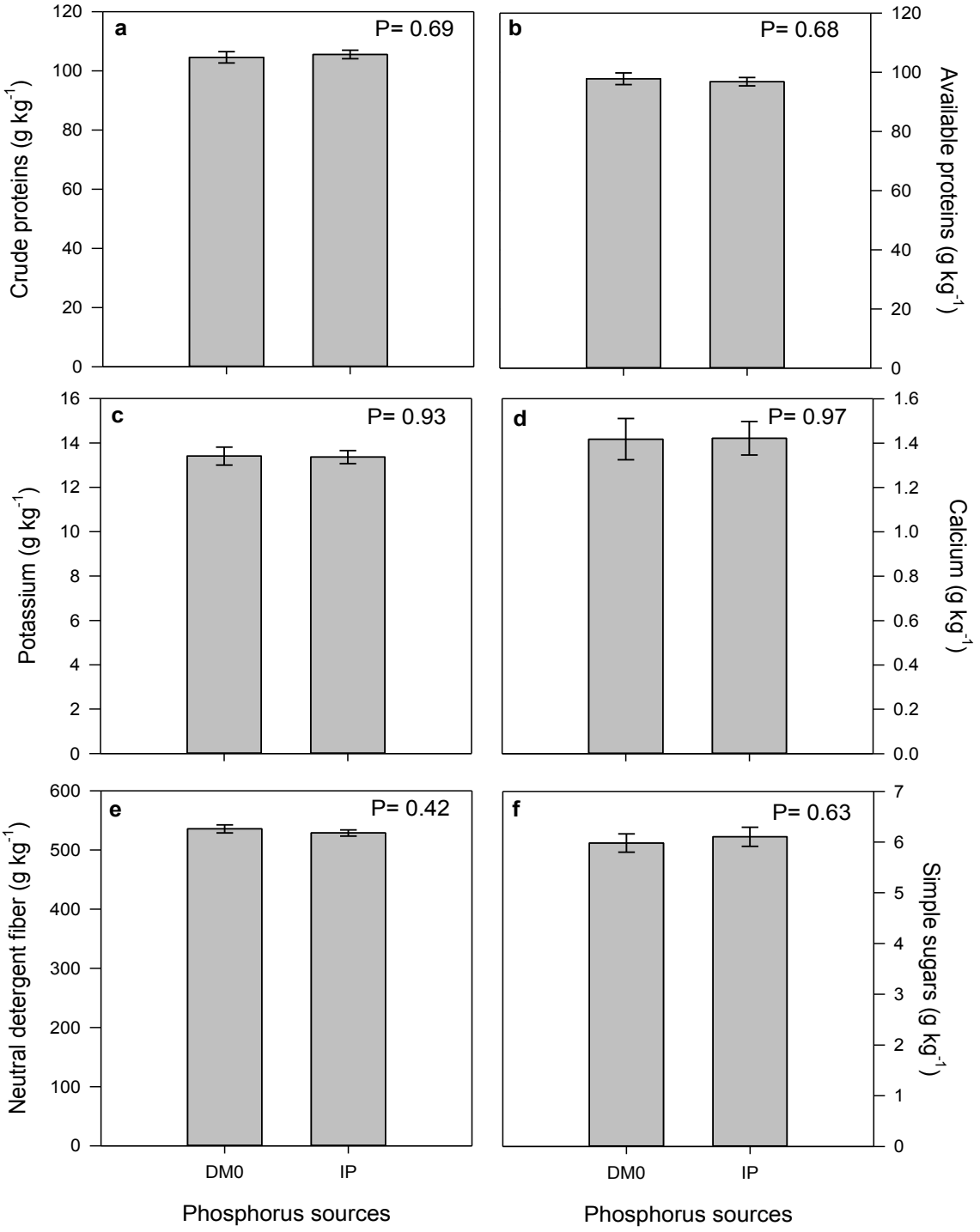

Figure 3.4.5 Quadrant 4 representing the effects of phosphorus sources on (a) crude proteins, (b) available proteins, (c) potassium, (d) calcium, (e) neutral detergent fiber and (f) simple sugars.

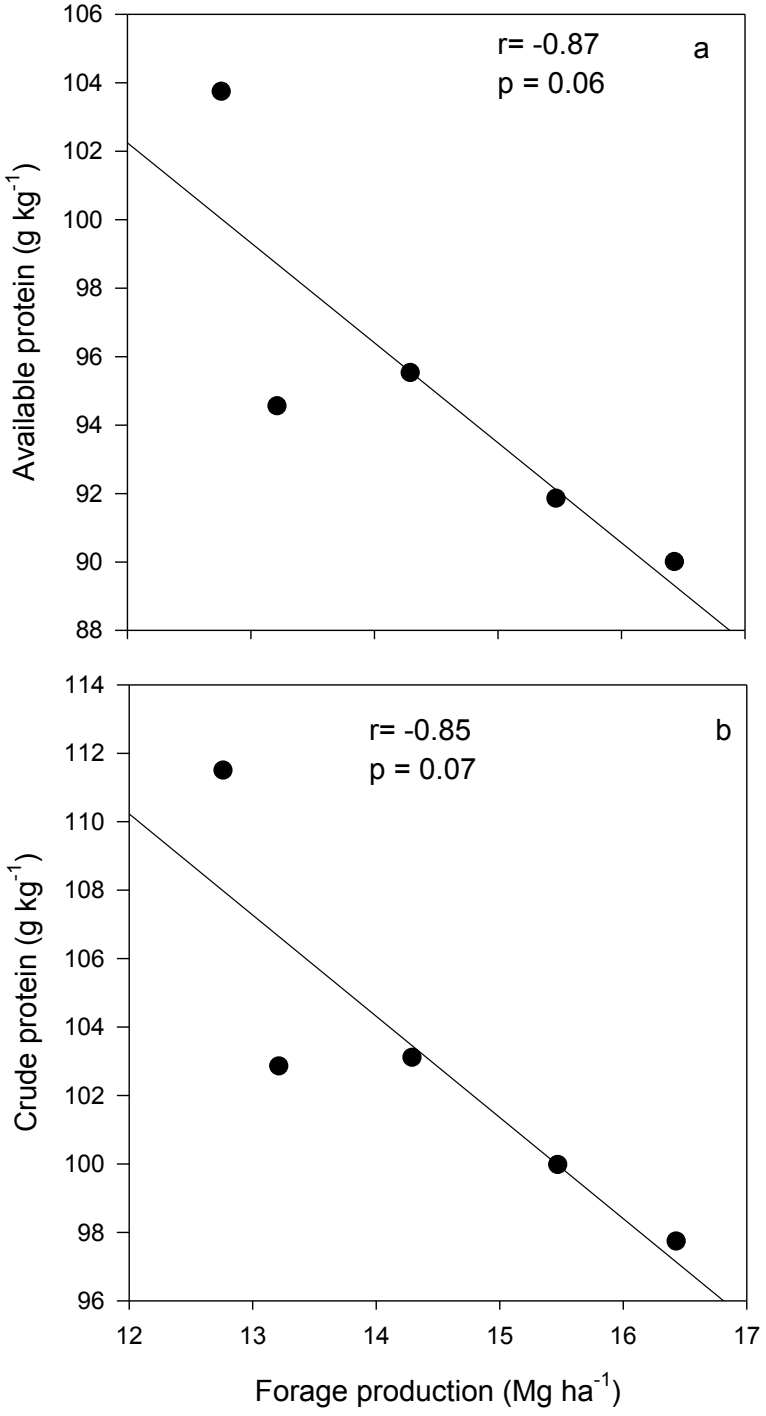

Figure 3.4.6 Pearson's correlation showing the association between forage production and forage proteins contents in five silage corn genotypes.

Table 3.3 Effect of silage corn genotypes on forage production and forage quality grown under cool climate in western Newfoundland, Canada.

Forage production and forage quality		Fusion RR	Yukon R	A4177G3RIB	DKC23-17RIB	DKC26-28RIB
Forage	Forage production (Mg ha^{-1})	14.29 ± 2.61^{b}	16.43 ± 2.38^{a}	12.76 ± 2.77^{c}	13.21 ± 3.29^{bc}	15.47 ± 2.66^{a}
Minerals	Phosphorus (g kg^{-1})	2.48 ± 0.36^{b}	2.60 ± 0.38^{ab}	2.75 ± 0.31^{a}	2.63 ± 0.29^{ab}	2.75 ± 0.32^{a}
	Calcium (g kg^{-1})	1.38 ± 0.49	1.18 ± 0.39	1.55 ± 1.11	1.48 ± 0.53	1.43 ± 0.48
	Potassium (g kg^{-1})	12.46 ± 1.77^{b}	12.66 ± 2.46^{b}	14.20 ± 1.69^{a}	12.56 ± 1.82^{b}	13.76 ± 2.59^{a}
	Magnesium (g kg^{-1})	1.26 ± 0.44^{c}	1.27 ± 0.43^{c}	1.71 ± 0.56^{a}	1.46 ± 0.33^{b}	1.35 ± 0.40^{bc}
Proteins	Crude protein (g kg^{-1})	103.11 ± 11.75^{b}	97.74 ± 12.29^{c}	111.51 ± 10.74^{a}	102.86 ± 11.02^{bc}	99.98 ± 10.12^{bc}
	Available protein (g kg^{-1})	95.53 ± 11.75^{b}	90.01 ± 11.33^{c}	103.75 ± 11.75^{a}	94.56 ± 11.14^{bc}	91.86 ± 9.36^{bc}
Sugar	Simple sugars (g kg^{-1})	6.60 ± 1.11^{a}	5.43 ± 1.00^{b}	5.64 ± 0.98^{b}	5.31 ± 0.86^{b}	6.32 ± 1.24^{a}
Fiber	Acid detergent fiber (g kg^{-1})	315.78 ± 29.63^{a}	305.39 ± 28.48^{ab}	298.44 ± 30.48^{b}	293.03 ± 32.78^{bc}	280.58 ± 32.12^{c}
	Neutral detergent fiber (g kg^{-1})	561.33 ± 38.34^{a}	528.33 ± 48.75^{b}	539.00 ± 39.51^{b}	524.28 ± 37.17^{b}	496.03 ± 43.89^{c}
NFC	Non fibrous carbohydrate (g kg^{-1})	262.03 ± 37.81^{c}	306.47 ± 47.24^{b}	280.50 ± 30.92^{c}	308.83 ± 38.24^{b}	345.22 ± 44.86^{a}
TDN	Total digestible nutrients (g kg^{-1})	636.11 ± 29.21^{a}	629.72 ± 18.28^{ab}	639.17 ± 26.01^{a}	617.22 ± 18.30^{c}	621.11 ± 19.97^{bc}
Energy	Net energy for lactation (Mcal kg^{-1})	1.38 ± 0.12	1.42 ± 0.11	1.41 ± 0.11	1.38 ± 0.08	1.41 ± 0.08
	Net energy for maintenance (Mcal kg^{-1})	1.39 ± 0.12^{a}	1.37 ± 0.08^{a}	1.40 ± 0.11^{a}	1.32 ± 0.07^{b}	1.33 ± 0.07^{b}
	Net energy for gain (Mcal kg^{-1})	0.81 ± 0.11^{a}	0.79 ± 0.07^{a}	0.82 ± 0.09^{a}	0.75 ± 0.07^{b}	0.75 ± 0.07^{b}

Values are means of three replications ± standard error. Mean values sharing different superscript in each row show significant differences among silage corn genotypes at alpha 0.05.

Table 3.4 Effect of phosphorus sources on forage production and forage quality in silage corn grown under cool climate in western Newfoundland, Canada

Forage production and forage quality		Control	DM1	DM2	IP
Forage	Forage production (Mg ha^{-1})	12.73 ± 2.51[b]	16.11 ± 3.29[a]	14.83 ± 3.03[ab]	14.51 ± 3.18[ab]
Minerals	Phosphorus (g kg^{-1})	2.60 ± 0.36	2.58 ± 0.35	2.68 ± 0.35	2.70 ± 0.32
	Calcium (g kg^{-1})	1.42 ± 0.62	1.43 ± 0.98	1.35 ± 0.39	1.42 ± 0.51
	Potassium (g kg^{-1})	13.41 ± 2.72	13.00 ± 2.06	12.75 ± 1.97	13.36 ± 1.96
	Magnesium (g kg^{-1})	1.47 ± 0.43	1.37 ± 0.52	1.41 ± 0.47	1.39 ± 0.43
Proteins	Crude protein (g kg^{-1})	104.61 ± 12.97[a]	99.92 ± 13.03[b]	102.07 ± 11.74[ab]	105.57 ± 9.60[a]
	Available protein (g kg^{-1})	97.83 ± 13.19[a]	92.27 ± 12.94[b]	93.62 ± 11.43[ab]	96.84 ± 9.41[a]
Sugar	Simple sugars (g kg^{-1})	5.98 ± 1.21[ab]	5.78 ± 1.19[ab]	5.59 ± 0.91[b]	6.10 ± 1.26[a]
Fiber	Acid detergent fiber (g kg^{-1})	303.31 ± 21.86	296.91 ± 34.48	295.89 ± 38.10	298.47 ± 25.28
	Neutral detergent fiber (g kg^{-1})	535.51 ± 45.84	526.73 ± 49.74	528.36 ± 53.88	528.58 ± 35.34
NFC	Non fibrous carbohydrate (g kg^{-1})	291.67 ± 50.96[b]	309.62 ± 51.71[a]	300.29 ± 53.82[ab]	300.89 ± 36.95[ab]
TDN	Total digestible nutrients (g kg^{-1})	628.22 ± 25.78	629.11 ± 23.72	630.67 ± 23.49	626.67 ± 23.84
Energy	Net energy for lactation (Mcal kg^{-1})	1.39 ± 0.11	1.41 ± 0.10	1.41 ± 0.11	1.39 ± 0.08
	Net energy for maintenance (Mcal kg^{-1})	1.36 ± 0.10	1.37 ± 0.09	1.37 ± 0.10	1.35 ± 0.09
	Net energy for gain (Mcal kg^{-1})	0.78 ± 0.09	0.79 ± 0.08	0.79 ± 0.09	0.79 ± 0.08

Values are means of three replications ± standard error. Mean values sharing different superscript in each row show significant differences among phosphorus sources at alpha 0.05.

3.4.2 Dairy manure amendment altered non-fibrous carbohydrates and net energy for lactation in silage corn (Q2)

PCA showed clear segregation of two silage corn genotypes (Yukon–R and DKC26–28RIB) and two P sources (DM_1 and DM_2) in quadrant–2 (Fig. 3.4.3). Biplot showed the relationship between non–fibrous carbohydrates (NFC), net energy for lactation (NEL) with Yukon–R, DKC26–28RIB, DM_1 and DM_2 (Fig. 3.4.1b). ANOVA showed that both genotypes (Yukon–R and DKC26–28RIB) expressed significant differences for NFC (Fig. 3.4.3a) and non-significant effects on NEL (Fig. 3.4.3c). NFC contents were significantly higher (348.22 g kg^{-1}) in DKC26–28RIB than Yukon–R (Fig. 3.4.3a). Keeping in view the grouping of manure sources and (DM_1 and DM_2), and both genotypes (DKC26–28RIB and Yukon–R) in the same quadrant (Q2), ANOVA was performed to determine the interactive effects of DM1, DM2 sources and control treatment with the two high biomass producing genotypes (Yukon–R as G2 and DKC26–28RIB as G5) on NFC and NEL parameters. The results showed non–significant effects of P sources and genotypes on NFC ($p = 0.16$ and p = 0.47; Fig. 3.4.3b) and NEL ($p = 0.84$ and $p = 0.25$; Figure 3.4.3d), although NFC contents were higher in G5 × DM2, compared to control and the other treatment combinations (Figure 3.4.3bd).

3.4.3 Forage production and ADF contents in silage corn genotypes (Q3)

Fusion–RR, DKC23–17RIB genotypes, forage production (FP) and acid detergent fiber (ADF) has been clustered the same quadrant–3 according to PCA (Figure 3.4.1ab). Keeping in view the relationship of DKC23–17RIB and Fusion–RR with ADF and FP in

the same quadrant, ANOVA was performed which showed that silage corn genotypes had significant ($p < 0.01$) effects on forage production and ADF contents (Fig. 3.4.4ab). It is evident that forage production was significantly ($p < 0.01$) higher in Yukon–R (16.43 Mg ha^{-1}) and DKC26–28RIB (15.47 Mg ha^{-1}), whereas the lowest was observed in A4177G3–RIB (12.76 Mg ha^{-1}). It also appeared that Fusion–RR (14.29 Mg ha^{-1}) and DKC23–17RIB (13.21 Mg ha^{-1}) produced forage in the intermediate range and therefore, were grouped in quadrant–3 (Figure 3.4.1a). Fusion–RR produced significantly ($p < 0.01$) higher ADF contents (315.78 g kg^{-1}), compared to lowest produced by DKC26-28RIB. However, Fusion–RR was statistically at par with Yukon–R (305.39 g kg^{-1}) and produced statistically similar ADF contents (Fig. 3.4.4b).

3.4.4 Effect of P sources on protein, minerals, fiber, and sugar contents in silage corn (Q4)

Protein (crude and available), minerals (Ca and K), simple sugars (SS), neutral detergent fiber (NDF) and IP source and control were clustered in Q4 (Fig. 3.4.1a &b). One–way ANOVA results revealed that IP and control treatments had non-significantly affected crude protein ($p = 0.69$), available protein ($p = 0.68$), K ($p = 0.93$), Ca ($p = 0.97$), NDF ($p = 0.42$), SS ($p = 0.63$) (Fig. 3.4.5a-f), and therefore, probably grouped in the same quadrant (Q–4).

3.5. Discussion

3.5.1. Mineral composition, net energy for maintenance and net energy for gain in silage corn genotypes (Q1)

Minerals play an important role in the maintenance, milk production, and energy provision processes in animals (Holtenius et al. 2008; Kronqvist 2011; Minson 1990; Spears 1994; Swift et al. 2007). Mg and P minerals are found in abundant in lactating cows (Kronquvist 2011; Plaizier et al. 2004), and are important part of bodily proteins, lipids and skeleton formation (McDowell 2003; Ravaglioli et al. 1996). Animals obtain most of the Mg and P from forages (Spears 1994; Suttle 2010). For example, Roth and Heinrichs (2001) observed 0.18% Mg and 0.23% P in silage corn forage; however, in the present study, we observed 0.13% – 0.17% Mg and 0.25% – 0.28% P in silage corn genotypes (Fig. 3.4.2). Our results also demonstrated higher Mg and P than reported by earlier researchers (Amodu et al. 2014; Roth and Heinrichs 2001; Suttle 2010). Littledike and Goff (1987) concluded that higher K concentrations in forage could result in lower Mg, whereas higher intakes of Ca and P in feed could result in suppressed Mg absorption in animals. However, we have observed Mg and P values in high quality forage range (Jacobson et al. 1972; Suttle 2010). Net energy for maintenance (NEM) and net energy for gain (NEG) represent the quality of forage species (Tine et al. 2001), and are calculated based on National Research Council (NRC) equations (2001) using summative approach of different quality parameters (Conrad et al. 1984; Schwab et al. 2003). Forage NEM keep the animals at a stable weight, whereas, NEG represents the energy for body weight gain required for

maintenance (Schwab et al. 2003). NEM (1.32 – 1.40 Mcal kg^{-1}) and NEG (0.75 – 0.82 Mcal kg^{-1}) observed in silage corn genotypes used in the present study were slightly lower than what observed by Ballard et al., (2001), who reported 1.46 – 1.68 Mcal kg^{-1} NEM and 0.88 – 1.06 Mcal kg^{-1} NEG of dry matter. This slight decrease in NEM and NEG might be due to crop harvesting stage where forage energy can be reduced due to increased forage fiber contents and slightly lower total digestible nutrients (TDN) (Di Marco et al. 2002; Peña-Ramos et al. 2002). Corn silage dry matter digestibility is an indication of energy contents or TDN, higher range of digestibility depends on hybrid, planting date, environmental growing conditions, and maturity at harvest (Guyader et al., 2018). Olagure-Ramirez et al., (2006) observed that good quality forage must contain TDN equal or greater than 65%; however, we observed slightly lower TDN (62% – 64%) among five genotypes (Table 3.3). This variation in TDN values could be due to crop harvest stage as reported by Kim et al., (2001), who observed higher TDN values in corn hybrids when harvested at 106–111 days after sowing (DAS) compared to 119–125 DAS due to increased forage fiber contents, whereas, in the present study, we harvested crop 130 DAS over three growing seasons. Manitoba Corn Committee conducted a varietal trials in 2017 and found that Yukon R and Fusion RR contains 68 and 66.1%. In present study, we found slightly lower values in Yukon and Fusion RR.

3.5.2. Dairy manure amendment altered non-fibrous carbohydrates and net energy for lactation in silage corn (Q2)

Non–fibrous carbohydrates (NFC), also referred as non-structural carbohydrates. NFC contents are more digestible than fiber, and a significant source of instant source of energy for rumen microbes (DePeters et al. 2000; Schwab et al. 2003). For example, during NFC fermentation process, volatile fatty acids are produced which are assimilated by the rumens and utilized as a source of energy for maintenance, synthesis of milk and body tissues (Harris 1993). NFC are calculated based on forage proteins, NDF, fats and minerals following equation suggested by NRC (2001). Observed NFC contents in our experiment were 34.5% in DKC26-28RIB and 30.6% in Yukon-R which were significantly higher (Fig. 3.4.3a & Table 3.3) than reported in the literature (Moreno-Reséndez et al. 2017). Higher NFC contents in our experiment explained the superior forage quality of silage corn genotypes cultivated in cool climate conditions. Moreno-Reséndez et al., (2017) reported higher NFC contents (31%) with inorganic fertilizer source compared to lower (21%) with organic source in forage maize. However, in our study DM application enhanced NFC content and were significantly higher with DM_1 application compared to control (Table 3.4). Slightly higher NFC contents in DKC26-28RIB × DM_2 interaction could be due to consistent P supply compared to control treatment, although interaction of Yukon-R, DKC26-28RIB with DM_1, DM_2 and control treatments were non-significant (Fig. 3.4.3b).

NEL is an important forage quality parameter estimated using TDN, CP, NDF and other forage quality parameters (Weiss et al. 1992). NEL is used for maintenance as well as milk production during lactation period (Schwab et al. 2003) and varied between 1.3 – 1.5 Mcal kg^{-1} of dry matter in silage corn (de la Cruz-Lázaro et al. 2007; Núñez-Hernández et al. 2004; Peña-Ramos et al. 2002). In present experiment, NEL in Yukon–R and DKC26–28RIB genotypes were 1.42 Mcal kg^{-1} and 1.41 Mcal kg^{-1} (Figure 3.4.3c). Moreno-Reséndez et al. (2017) observed, that inorganic nutrient sources resulted in significantly higher NEL (1.05 Mcal kg^{-1} of dry matter) than organic nutrient sources (0.98 Mcal kg^{-1} of dry matter). However, we observed non-significant interactive effects of two high forage yielding genotypes with DM and control on NEL (Fig. 3.4.3d).

3.5.3. Forage production and ADF contents in silage corn genotypes (Q3)

Yukon-R and DKC26-28RIB showed superior agronomic performance and produced 29% and 21% higher forage yield compared to the lowest produced by A4177G3–RIB (Figure 3.4.4a). Interestingly, Yukon–R and DKC26–28RIB required same CHU (2150) to reach the physiological maturity that might have stimulated better growth, development and final forage production compared to other genotypes with different CHU requirements (2075 vs. 2200). Kwabiah (2005) observed significant differences in biomass production (9-13 Mg ha^{-1}) among three silage corn genotypes while conducting field studies in similar soil and environment conditions whereas, forage yield in present three years' field experiment varied from 12.76 Mg ha^{-1} to16.43 Mg ha^{-1} which was significantly higher than reported above. Variation in forage production apparently under similar

weather and soil conditions could be due to better resilience, adaptation and genetic make-up of silage corn genotypes used in the present experiment.

P sources had significant effects on forage production of silage corn (Table 3.4). For example, DM_1 treatment produced significantly higher forage production compared to control, however, DM_1 treatment was statistically at par with IP and therefore, gave similar forage yield despite lower nutrient supply in the readily available form resulting from slow release from DM, may be due to the soil amelioration effects of DM on physicochemical and biological properties of soil associated with better nutrient uptake efficiencies and optimum plant growth as suggested by Adeli et al., (2007), Hirzel et al., (2007b) and Nazli et al., (2016). Furthermore, DM/organic materials also stimulate root growth directly or indirectly or through their effect on soil bacteria that can suppress root pathogens and produce plant growth hormones (Marschner, 1995; Palm et al., 1997).

ADF is comprised of cellulose and lignin (Moreno-Reséndez et al. 2017) and is indicator of good forage quality. Increased ADF result in low forage digestibility, energy contents and intake potential (Castillo-Jiménez et al. 2009; Gallegos-Ponce et al. 2012; Oramas-Wenholz and Vivas-Quila 2007; Pinkerton and Cross, 1992). Forage is considered of optimum quality if ADF contents are lower than 30% (Gallegos-Ponce et al. 2012). Significant variations in ADF (24.70% – 30.80%) were observed in two silage corn hybrids when harvested 119 DAS (Amodu et al. 2014), and 23.20% – 26.00% were observed when harvested 131 DAS (Ballard et al. 2001). The observed ADF contents in the present study varied between 28.06% - 31.58%, and were higher in Fusion-RR and lower in DKC26-28RIB (Fig. 3.4.4b & Table 3.3), and can be considered as superior quality forage

(Gallegos-Ponce et al., 2012). In a varietal research trial conducted by Manitoba Corn Committee during 2017 and evaluated the quality of various hybrids, and found that Yukon R and Fusion RR contains 27 and 30.5% ADF contents (Manitoba Corn Committe, 2017). Significant differences in ADF contents were observed when amended with organic or inorganic nutrient sources (Moreno-Reséndez et al. 2017; Nazli et al. 2016). The N-based organic material application gave the highest ADF contents, whereas, P based organic treatments gave similar ADF contents to inorganic treatment. This could be attributed to differences in N contents among these treatments because they strongly influence fiber content as well (Keeney et al., 1968; Cherney and Cox, 1992; Johnson et al., 2001). In the present study, we have also observed non-significant effects of DM and IP sources on ADF contents (Table 3.4). Consistent with our findings, non-significant effects of P based cattle manure, poultry litter and inorganic fertilizer on ADF were observed (Nazli et al., 2016).

3.5.4. Effects of P sources on protein, minerals, fiber, and sugar contents in silage corn (Q4)

Proteins are organic compounds and are important animal feed component. RNA (ribonucleic acid) play very vital role in protein synthesis, for example, messenger RNA (mRNA), ribosomal RNA (rRNA), and trafner RNA (tRNA) are involved in protein synthesis in the cytosol, chloroplasts and mitochondria (Raven, 2013). Phosphorus is a component of the complex nucleic acid structure of plants, which regulates protein synthesis. Phosphorus is, therefore, important in cell division and development of new tissues (Raven, 2013).

To qualify a good forage, crude protein (CP) must be higher than 7% to guarantee adequate supply of nitrogen (N) for effective rumen microbial fermentation (Costa et al. 2006; Garibay et al. 1997; Oramas-Wenholz and Vivas-Quila 2007). Previous researchers found significant variations in protein contents in silage corn, for instance, 7.10% – 7.50% (Baron et al., 2006), 5.20% - 6.50% (Amodu et al., 2014), 6.60% - 6.80% (Ballard et al., 2001) and 6.60% - 7.25% (Millner et al., 2005). However, observed CP contents in the present study were 9.77% – 11.15% in silage corn genotypes (Table 3.3), and were 36% higher than reported in the literature (Ballard et al. 2001; Baron et al. 2006; Darby and Lauer 2002; Garibay et al. 1997; Moreno-Reséndez et al. 2017). A strong negative correlation between forage production and available protein (AP) ($r = -0.87$), and CP ($r = -0.85$) was noted in the present study (Figure 3.4.6ab), which suggest some possible N dilution effects in high yielding silage corn genotypes (Bélanger and Gastal 2000; Fletcher and Chakwizira 2012).

Few studies reported inorganic P fertilizers enhanced forage protein in silage corn compared to organic source or dairy digestate (Faisal et al., 2013; Seleiman et al., 2017). This could be explained by lower nutrient supply in the readily available form to plants resulting from slow release of mineral nutrients from DM. In present study, inorganic P fertilizer source produced higher CP contents than DM_1 but statistically at par with DM_2 and control (Table 3.4). Interestingly, we have observed non-significant effects of IP and control treatment on CP and available protein (AP) that could be explained due to high initial P status in control, therefore, IP and control produced statistically similar protein contents.

The performance of dairy animals is dependent on adequate supply of forage minerals which may vary due to soil fertility, fertilization strategies and crop maturity (Holtenius et al. 2008; Swift et al. 2007). Calcium (Ca) is an integral part of animal body and play essential role in bones formation, normal muscle contractions and nerves functioning (Goff, 2008; Kronqvist, 2011). Calcium react with P and forms Ca-hydroxyapatite, which are an important part of skeleton in animals body (Kronqvist, 2011). K is principal intercellular cation of body tissues and plays key role in many biological processes (Bannink et al., 1999). Corn forage is generally low in Ca (1.4 g kg^{-1} – 3.0 g kg^{-1}), whereas, K ranged up to 1.23% on dry weight basis (Suttle, 2010). Minimum Ca and K requirements for lactating cows vary from 0.29% to 0.51% and 0.60% to 0.80% respectively, on a dry weight basis (Suttle, 2010). In present three years study, we found that Ca range between 1.18 – 1.55 g kg^{-1} in silage corn genotypes (Fig. 3.4.5d). In a greenhouse study, it was reported that among P sources (mono-ammonium phosphate (MAP), compost and dairy manure), Ca uptake in silage corn increased with inorganic P source (mono-ammonium phosphate-MAP) application, while the manure and compost treatments showed non-significant difference. Reduction of Ca uptake in silage corn under manure and compost treatment could be related to cation competition with K (Leytem et al., 2011). In the present studies, DM and inorganic P sources had non-significant effects on forage Ca in silage corn (Table 3.4), even IP treatment and control produced similar Ca in silage corn (Figure 3.4.5d), might be due to accumulation of P over the time which raised initial soil P status in control.

NDF comprised of hemicellulose, cellulose and lignin representing the fibrous bulk of the forages, and these three components provide rigidity to plants cells. NDF in forage crops are negatively correlated with intake and digestibility (Oramas-Wenholz and Quila, 2007), so silage corn hybrids less than 50% NDF are needed (Gallegos-Ponce et al., 2012). Fiber concentration of corn silage typically decreased with maturity stage due to dilution effects of increasing starch concentration (Hunt et al., 1992; Russell, 1986). NDF and starch contents usually showed negative relationship with each other. Late planting date may increase NDF digestibility, as plants are less mature at harvest. Late sowing in short season areas limits corn heating unit (CHU) accumulation, and hence limits dry matter yield, starch content and dry matter digestibility (Darby and Lauer, 2002). Few researchers reported that N-based organic material applications gave the highest NDF contents, whereas, P based poultry and cattle manure treatments gave similar NDF contents to IP. This could be attributed to differences in nitrogen content among these treatments because that strongly influences fiber content as well (Keeney et al., 1968; Cherney and Cox, 1992; Johnson et al., 2001). In the present study, non-significant effects of DM and IP sources, and IP and control on NDF contents were reported (Fig. 3.4.5e) which are inconsistent with the studies reported above. Same NDF contents in IP vs. control treatment could be explained due to higher initial soil P status in analysis report and therefore, response to NDF contents was non-significant.

3.6. Conclusion

Sustainable agricultural production practices such as use of organic fertilizer source includes DM to produce high forage biomass with optimum nutritional quality is important under depleting inorganic P resources worldwide. High production and high forage quality is of prime importance for high gain and ample milk production in the dairy industry. Silage corn is one of the most important forage crop grown for higher forage production potentials, high energy content, palatability and digestibility compared to other forage species. We used DM and IP fertilizer to determine the production potential and forage quality of five silage corn genotypes in cool climate production systems. Silage corn genotypes were grouped in three quadrants (Q1, Q2, and Q3) and P sources in two quadrants (Q2 and Q4) (Fig. 1a). Yukon-R and DKC26-28RIB showed superior agronomic performance and produced significantly higher forage production of 16.43 and 15.47 Mg ha^{-1} respectively. Additionally, P, K, simple sugars, and NFC were high and ADF and NDF contents were low in Yukon–R and DKC26–28RIB compared to the other genotypes, and are indicators of high forage quality. A4177G3–RIB genotype produced minimum (12.76 Mg ha^{-1}) forage and was statistically similar with DKC23–17RIB. However, this genotype produced forage with superior nutritional quality. For instance, P, K, Mg, CP, AP, TDN, NEM and NEG was significantly higher in A4177G3–RIB compared to the rest of genotypes. Yukon–R also produced high forage quality and was closest to that produced by A4177G3–RIB genotype. Dairy manure with high P concentration (DM1) significantly enhanced agronomic performance and produced higher forage biomass compared to control. Forage quality characteristics, for example, protein and sugar contents were higher

in IP treatment compared to the control and DM2 treatment; but at par or very close to DM1 treatment which suggest that dairy manure application could be a sustainable agricultural practices or P nutrient source. Taking all together, it can be concluded that Yukon–R and DM1 can be a good option to attain high forage production and forage quality to meet the forage needs of growing dairy industry in northern climates.

3.7. References

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4. Chapter 4

4.1. General Discussion and Conclusion

Our objectives were to evaluate the effects of organic and inorganic P sources and genotypic response on;

i. Agronomic performance (leaf area, leaf chlorophyll contents, net photosynthesis and dry matter yield) of silage corn.
ii. Soil available P, enzyme activities, and microbial communities' abundance.
iii. Relationship between agronomic performance, biochemical attributes and active microbial communities.
iv. To investigate the effects of phosphorus sources on production potential and quality of silage corn genotypes.

These objectives were achieved through two main experimental works as described in Chapter 2 and 3. Effects of phosphorus sources on agronomic performance of silage corn genotypes, soil biochemical attributes and soil microbial phospholipid fatty acids have been described in chapter 2. Whereas, effects of P sources on production potential and quality of silage corn genotypes were discussed in chapter 3.

4.2. Effects of organic and inorganic phosphorus sources on agronomic performance of silage corn

4.2.1. Leaf area

Leaves play very important role in the photosynthesis and other physiological processes which may increase dry matter production (Khan and Khalil, 2010; Man et al., 2015). Phosphorus deficiency results in stunted growth in young plants and a dark green coloration of leaves (Chen et al., 2014). It reduce 67 % mean leaf area, and 43 % leaf emergence, and significant reduction in intercepted solar radiation, photosynthesis and dry biomass production (Fredeen et al., 1989; Plenet et al., 2000). In 2016, leaf area of Yukon R corn genotype significantly increased with DM application as compared to control treatment and this increment was attributed to optimum supply of nitrogen (N) and P to the plants (Hariadi et al., 2016). Whereas, during 2017, non-significant trends observed for G x P interaction, that might be due to less rainfall (Table 2.2). Our results of 2017 growing season are quite in line with that of Song et al., (2018) who reported that soil moisture deficit at vegetative stage significantly limit crop growth and leaf area of corn.

4.2.2. Chlorophyll contents

Chlorophyll is a green pigment and involves in energy harvesting reaction that can be used to assimilate carbon dioxide and convert absorbed light into chemical energy for photosynthesis. Decomposition and mineralization of organic matter content present in DM release organic acids which play an important role in the supply of Fe^{2+}, Mg^{2+} and

NH^{4+}, which are necessary for enzyme activation, chloroplast and chlorophyll formation (Elhindi, 2012; Hasan et al., 2014). DM application significantly increased chlorophyll contents of maize as compared to control (Efthimiadou et al., 2009). Results of present study also report that DM application either with high or low P and or N concentration significantly increased chlorophyll contents of Yukon R and DKC26-28RIB compared to control during both years (2016 and 2017 growing seasons). This increase could attributed to optimum supply of N and Mg^{+2} from DM (Table 2.5) that might have played an important role in chlorophyll synthesis, as both compounds are central component of chlorophyll molecules. Manure application improved optimum supply of N and Mg^{+2} to crop plants which may have led to increase chlorophyll contents of plants because both compounds are central component of chlorophyll molecules. Our results are in line with previous findings (Elhindi, 2012) which reported that manure application significantly improved chlorophyll contents of crop plants presumably by increasing availability of N and Mg^{2+} in soil.

4.2.3. Photosynthesis rate

Phosphorus is an important element in compounds such as ATP, NADPH, nucleic acids, sugar phosphates, and phospholipids which are involved in photosynthesis (Hammond and White, 2008). Photosynthesis is a process in which plants absorbs light energy and convert in to chemical energy (Hohmann-Marriott and Blankenship, 2011). P deficient plants showed reduction in photosynthesis that might be due to diminishing of ribulose 1-5, bisphosphate (RuBP) generation rather than due to limitations in the supply

of ATP (adenosine triphosphate) and NADPH (Nicotinamide adenine dinucleotide phosphate oxidase) in the Calvin cycle (Fredeen et al., 1989). RuBP is formed by taking a phosphate coming from the splitting of ATP, and joining it with riblulose phosphate (RuP), changing RuP into RuBP, which is then able to react with CO_2 and form an unstable 6C (carbon) molecule which is the basis for the dark reaction. ATP is commonly known as energy currency of the cell because energy obtained through its metabolism is used for biomolecules synthesis, movement and cell division. ATP consists of the purine base adenine, pentose sugar ribose, and three phosphate groups, and anhydrous bonds of the three phosphate make ATP a high energy molecule. During the photosynthetic electron transfer reaction H^+ pumped across the thylakoid membrane, and the resulting electrochemical proton gradient derives the synthesis of ATP in the stroma (Alberts et al., 2002). APT hydrolysis produces more free energy compounds such as ADP (adenosine diphosphate) and inorganic phosphate than that of other phosphate compounds and common phosphate ester (Alberts et al., 2002). As the final step of electron transport chain, high energy electrons are loaded (together with H^+) on to $NADP^+$, converting it to NADPH, and all of these reactions are confined to chloroplast (Alberts et al., 2002). In carbon fixation process, three molecules of ATP and two molecules of NADPH are consumed for each molecule of carbon dioxide (CO_2) converted in to carbohydrate. The net equation is: $3C02 + 9ATP + 6NADPH + water \rightarrow Glyceraldehyde\ 3 - phosphate + 8P_i + 9ADP + 6NADP^+$(Alberts et al., 2002). Orthophosphate in the chloroplast stroma serve as a substrate for ATP synthesis (Carstensen et al., 2018).

Recent work exhibited that P deficiency reduces the orthophosphate concentration

(Pi) in the chloroplast stroma which might have led to inhibit the process of ATP synthesis (Carstensen et al., 2018; Karlsson et al., 2015), and protons start accumulating in the thylakoids and cause lumen acidification, which stops linear electron flow. Inadequate plastoquinol (PQH2) oxidation retards electron transport to the cytochrome *b6f complex*. However, the electron transfer rate of PSI is increased under steady state growth light conditions and is limited under high light conditions. P deficiency increased electron transport through PSI increases the levels of NADPH, whereas ATP production remains limited and reduced CO_2 assimilation. Changes in supply of Pi in the stroma reduced the ATP levels and consequently reduced, but no changes in the photosynthetic machinery composition were observed (Karlsson et al., 2015). P deficiency immediately affects CO_2 fixation, but does not appear to stop it, as P deficient plants typically remains green and do not develop leaf chlorosis. Phosphorus starvation significantly reduced $NADP^+$ in to NADPH, indicating that a large fraction of $NADP^+$ remains in the reduced form NADPH, because it cannot be utilized in the Calvin cycle due to ATP limitation, and because of higher PS1 activity (Carstensen et al., 2018). NADPH concentration was significantly higher under P deficient treatment as compared to P supply treatments.

DM application significantly improved photosynthesis rate and plant growth compared to barley mulch, poultry manure and mineral fertilizers was attributed to optimum and slow release of nutrients (Efthimiadou et al., 2009; Salehi et al., 2017). In the present study, results described that manure with high and low P concentration significantly increased the photosynthesis rate of Yukon R and DKC26-28RIIB compared to control during 2016. Genotypes x Phosphorus sources interaction was non-significant in 2017 that

might be due to less rainfall possibly caused the reduction in macro and micro nutrients uptake. In 2017 vs 2016, drought stress due to insufficient water supply and rainfall led to closure of plants stomata to prevent the water loss by transpiration (Yan et al., 2011; Zhang et al., 2012) and consequently, affected photosynthesis rate due to decreased level of CO_2 assimilation and phosphorus uptake at chloroplast level (Carstensen et al., 2018; Long et al., 2006).

4.2.4. Root shoot ratio

Plant species has shown adaptations under P deficient conditions; one by increasing length and density of lateral root hairs in corn and many other plant species which would improve P uptake through expansion of root surface area at minimal cost (Lynch and Brown, 2008). Another adaption to P deficiency includes increase in root/shoot ratio, root branching, elongation, top foraging and cluster root hairs possibly due to assimilates distribution (Lynch and Brown, 2008; Wissuwa et al., 2005). P starvation caused changes in carbohydrates distribution between roots and shoots (Wissuwa et al., 2005). A larger root system has been recognized as an important adaptation in plants to cope water and nutrient stress (Wissuwa et al., 2005). In present study, we found that root to shoot ratio of silage corn genotypes were significantly higher under control, and this increased might be due to distribution of carbohydrates and P from shoots to roots may helpful for plants to adapt low P stress condition which may account for increase in root to shoot ratio. Our findings are in agreement with the earlier studies (Basirat et al., 2011; Li et al., 2009; Louw-gaume et al., 2010; Wissuwa et al., 2005) where authors suggested that P deficiency

significantly increased root to shoot ratio of crops. Corn cultivars exhibited significant difference in root length due to variation in cultivar variability (Szoboszlay et al., 2015).

4.2.5. Plant height

Short plant height in control (without fertilizers/manure application) might be due to leaching/depletion of nutrients over the time, hence plants height was reduced due to insufficient nutrients supply. DM application significantly increased plant height of corn through optimum supply of P and other nutrients which might increase nodes and internode length formation (Amujoyegbe et al., 2007; Efthimiadou et al., 2009). Increase in Yukon R plant height with application of manure compared to control was attributed to optimum supply of nutrients through manure. Our results supported by (Efthimiadou et al., 2009), who found that manure application produced positive influence on plant height of corn compared to other inorganic fertilizers. Highest plant height noted in Yukon R might be due to cultivar variability among the genotypes (Maryam et al., 2012), and slow release of nutrients with DM application throughout the growing season. Reduction in plant height during 2017 growing season might be due to less rain caused water stress which might lead to stunted growth by ceasing cell division and elongation processes (Maryam et al., 2012).

4.2.6. Dry matter yield

Dry matter accumulation by crops depends upon interception of photosynthetically active radiation and efficiency of leaves to convert intercepted light in to dry matter yield (Portes and Melo, 2014). Results of present study explained that DM with high P

application significantly increased leaf area, chlorophyll contents, photosynthesis rate and plant height which consequently led to increased dry matter yield compared to control. Higher dry matter yield and yield components in DM amended treatment could be attributed to optimum supply of N, P and other macro and micro-nutrients which improved the crop growth and dry matter yield throughout growing season. Our results are in line with the findings of (Amujoyegbe et al., 2007; Efthimiadou et al., 2009), who reported that manure application significantly improved leaf area, chlorophyll contents photosynthesis and dry matter yield of silage corn as compared to inorganic fertilizer. Salehi et al. (2017) found that dry matter yield was significantly increased with manure application due to gradual release of nutrients that improved photosynthesis rate and plant growth. Less rainfall during 2017 (Table 2.2), caused significant reduction in leaf area, chlorophyll contents, plant height, photosynthesis and dry matter yield of silage corn (Abrokwah et al., 2017; Bouazzama et al., 2012; Maryam et al., 2012; Yan et al., 2011).

4.3. Effects of P sources on soil biochemical attributes

4.3.1.1. Soil rhizosphere pH

Rhizosphere soil pH is an important factor that affects the soil biochemical properties (Gregory and Hinsinger, 1999), and determine the availability of macro and micro nutrients (Hinsinger, 2001). Manure and organic fertilizers application enhance soil pH and soil organic carbon (SOC) than synthetic fertilizers (Dong et al., 2012; Lapa et al., 2011). In present study, we found that soil rhizosphere pH was slightly higher in DM amended treatments and inorganic P treatments than control (Table 2.18). Earlier studies

also reported that manure application stabilize or increased soil pH (Dong et al., 2012; Whalen et al., 2000). Difference in soil rhizosphere pH of genotypes reported in present study might be due protons secreted by roots in response to uptake of cations and anions by plant species (Hinsinger, 2001; Li et al., 2008).

4.3.1.2. Acid phosphatase activity

Soil enzymes activities are important indicator of soil health, and play important role in organic matter decomposition and nutrients turnover (Paz-Ferreiro et al., 2007). Enzymes activities may fluctuate rapidly due to fertilizer sources, complex rhizosphere processes, biological properties, soil physio-chemical properties and environmental conditions (Paz-Ferreiro et al., 2011). Organic P present in manure is mineralized by acid and alkaline phosphatase enzyme into plant available P forms (Waldrip et al., 2012). Inorganic P fertilizers application significantly decreased the phosphatase activity, because plants and microorganisms easily obtain P from readily available sources (Spohn and Kuzyakov, 2013). Our results have demonstrated that DM application significantly increased acid phosphatase activity compared to inorganic P fertilizer. Higher amount of C, N and P along with trace elements in DM increased microbial activity which consequently increased enzymes activities (Li et al., 2016; Zhang et al., 2015). Silage corn genotypes exhibited difference in acid phosphatase activity in their rhizosphere, which might be due to cultivar variability of roots to secrete acid phosphatase. Our findings are in line with earlier researchers results (Colvan et al., 2001; Leytem et al., 2011; Waldrip et

al., 2012) who reported that manure application significantly increase acid phosphatase activity, whereas inorganic P application reduced acid phosphatase activity.

4.4. Soil microbial phospholipids fatty acids (PLFAs) profile

PLFAs are widely used as soil microbial biomarkers and indicate viable components of soil microbial biomass, and provide more detailed information about 'active' soil microbial community compared to culture method (Liang et al., 2008; Yao et al., 2000). In agroecosystems, organic and inorganic fertilizers are applied to enhance nutrients availability to crops but they also affect the soil microbial communities (Guo and Wang, 2009; Wei et al., 2017; Zhang et al., 2015). External P fertilization significantly increased soil microbial diversity and abundance in forest, grassland and pastures (Liu et al., 2013; Pan et al., 2014), specifically Gram negative (G^-) bacteria, arbuscular mycorrhizal fungi (AMF), total bacterial and total amount of soil PLFAs compared to control, whereas mean abundance of gram positive (G^+) bacteria did not differ among treatments (Liu et al., 2013). Significant changes were observed in microbial communities of two maize genotypes rhizosphere most likely due to secretion of root exudates such as sugars, amino acids, organic acids and hormones that might have enhanced bacterial growth in soil rhizosphere and higher availability of C in manure amended treatment (Wang et al., 2017). Agricultural soils are generally carbon limited and DM amendment stimulate soil microbial communities by increasing SOC labile fractions, N and P pool in soil, which probably could serve as major energy sources for microorganisms (Demoling et al., 2007). In current study, we found that DM application significantly increased active

G^- bacteria, bacterial PLFAs, microbial biomass (total amount of PLFAs), and fungal PLFAs compared to control. Wei et al. (2017) reported that manure application significantly increased active G-, G^+ bacteria, active fungal PLFAs, and microbial biomass (total amount of PLFAs). Our findings of G^+/G^- ratio are in line with Wei et al. (2017), who reported that changes in G^+ and G^- bacterial community were strongly related to increase in soil organic C added by application of manure. In general a lower G^+/G^- ratio is an indicator of better soil nutrition (Rajendran et al., 1997).

Fungi involves in C and nutrients turnover in agroecosystem and are sensitive to external fertilizers application (Li et al., 2018). In present study, we found that DM with high P concentration significantly increased active fungal PLFAs compared to control, which could be attributed to additional SOC attained by DM application and served as major source of energy for active fungi whereas, small increase in labile organic C under inorganic fertilization and control treatments reduced growth (Liu et al., 2013). Higher F/B ratio can reflect the relative abundance of microbial population which is an important indicator of a strong soil ecosystem buffering capacity and more sustainable land use (De Vries et al., 2006). Our results showed that F/B ratio significantly increased by manure application in 2017 compared to 2016. This difference in F/B ratio might be due to higher organic matter contents (3.01%) in soil and furthermore manure applied in soil contains higher percentage of P, N and C in P_1 manure treatment during 2017 relative to 2016 organic matter contents (2.98%) (Table 2.1 and 2.5), and fungi may play a larger role in organic matter decomposition and C cycling (Buyer et al., 2010; De Vries et al., 2006; Liu et al., 2013).

Various plant species and genotypes roots generally release organic acids, sugars, amino acids, lipids, enzymes and aromatic compounds which in turn stimulate the growth of dormant microbial species (Li et al., 2014; Szoboszlay et al., 2015). In present study, Yukon R exhibited higher bacterial PLFAs, microbial biomass (total amount of PLFAs) and active fungal PLFAs, which might suggest that Yukon R presumably released more diverse and abundant secondary metabolites which in turn enhanced microbial population.

4.5. Effects of P sources on quality indices of silage corn

Corn growing for silage is considered to be the most suitable crop as compared to other cereals due to fast growth, higher yield potential, higher palatability, energy contents, protein contents, sugar and water soluble carbohydrate which are most important in the preservation of silage material (Amin, 2011; Keady et al., 2008). Good quality silage corn contains 28-32% dry matter yield, 28-32% starch and 7-9% crude protein, and addition of silage corn based diets of dairy cows increases feed intake, milk yield and milk protein content (Keady et al., 2008; Kwabiah et al., 2003; Ullah et al., 2015). As a result silage corn becomes the major feed component in the ration of dairy cows under most dietary regimes (Keady et al., 2008). Dairy industry is heavily relying on silage corn for dairy animals, therefore, it is important to assess the nutritional quality of silage corn in cool climate amended with organic and inorganic P sources.

4.5.1. Mineral composition

Minerals play an important role in milk production, reproduction and energy provision in animals (Holtenius et al., 2008; Kronqvist, 2011), essential role in skeleton formation, protein and lipids synthesis (Kronqvist, 2011; Plaizier et al., 2004), and fulfill through forage feed (Suttle, 2010). For example, Mg and P concentration in forages vary between 1.8 – 3.6 g kg^{-1} and 1.8 – 3.3 g kg^{-1} of dry matter respectively, and significantly affected by forage type, growth conditions, harvesting stage, P source and rate of P application (Kronqvist, 2011; Leytem et al., 2011; Suttle, 2010). Silage corn contains 0.18% Mg and 0.23% P (Roth and Heinrichs, 2001). Leytem et al. (2011) conducted a greenhouse study to determine the effects of phosphorus sources (mono-ammonium phosphate (MAP), compost and dairy manure) and rates on nutrients uptake in silage corn. P, Ca uptake in silage corn increased with inorganic P source (mono-ammonium phosphate-MAP) application, while the manure and compost treatments showed non-significant difference. Manure and compost contains iron which might have reacted with P and forms Fe phosphates thereby reducing P solubility. Reduction of Ca uptake in silage corn under manure and compost treatment could be related to cation competition with K (Leytem et al., 2011). Magnesium uptake increased under MAP treatment and reduced for manure (Leytem et al., 2011). High K uptake might have inhibited Mg uptake due to cation competition and balance between K, Ca and Mg uptake could be a concern from an animal health prospective as forages with K:(Ca+Mg) ratios greater than 2.2:1 cause grass tetany in ruminant (Grunes et al., 1970). In current study, we found that Mg ranges between 0.13

- 0.17% and P (0.25 – 0.28%) in silage corn genotypes (Table 3.3), and was slightly higher than earlier studies (Amodu et al., 2014; Roth and Heinrichs, 2001; Suttle, 2010).

Calcium (Ca) is an integral part of animal body and play essential role in bones formation, normal muscle contractions and nerves functioning (Goff, 2008; Kronqvist, 2011). Calcium react with P and forms Ca-hydroxyapatite, which are an important part of skeleton in animals (Kronqvist, 2011). K is a principal intercellular cation of body tissues and plays key role in many biological processes (Bannink et al., 1999). Corn forage is generally low in Ca (1.4 g kg^{-1} – 3.0 g kg^{-1}), whereas, K ranged up to 1.23% on dry weight basis (Suttle, 2010). Minimum Ca requirements for lactating cows ranged from 0.29% to 0.51% whereas K ranged from 0.60% to 0.80% on a dry weight basis (Suttle, 2010). In present three years study, we found that Ca range between 1.18 – 1.55 g kg^{-1} in silage corn genotypes, whereas organic and inorganic P sources exhibited non-significant difference in Ca uptake in silage corn.

4.5.2. Crude and available protein

Proteins are organic compounds, composed of amino acids and are important animal feed component. RNA (ribonucleic acid) is a polymer molecule, consisted of ribose sugar, nucleotide bases and phosphate group. RNA play very vital role in protein synthesis, for example, messenger RNA (mRNA), ribosomal RNA (rRNA), and trafner RNA (tRNA) involve in protein synthesis in the cytosol, chloroplasts and mitochondria, with 70S ribosomes in the organelles and 80S ribosomes in the cytosol (Raven, 2013). Phosphorus is a component of the complex nucleic acid structure of plants, which regulates protein

synthesis. Phosphorus is, therefore, important in cell division and development of new tissue. Organic fertilizers are good source of N, P and micro-nutrients but generally they are slowly available to plants, whereas macro and micro-nutrients are readily available to the plants by inorganic fertilizer application which may affect the composition of forage quality. Conversely, Moreno-Resendez et al. (2017) reported increased protein in forage treated with organic source compared to inorganic fertilizer application. In present study, we found that organic and inorganic P fertilizer application produced similar protein contents in silage corn, which suggest that DM application could be a sustainable approach for obtaining optimum CP contents in silage corn and may reduce dependence on inorganic P fertilizers.

Protein contents in silage corn vary between 7.10% – 7.50% (Baron et al., (2006), 5.20 - 6.50% (Amodu et al., 2014), and 6.60 - 7.25% (Millner et al., 2005). However, in good quality forage, crude protein (CP) should be more than 7% (Garibay et al., 1997; Oramas-Wenholz and Quila, 2007). CP contents in the present study were > 7% (Table 3.3), and 36% higher than reported in the literature (Ballard et al., 2001; Baron et al., 2006; Garibay et al., 1997; Moreno-Resendez et al., 2017) and therefore, can be considered as high quality forage (Garibay et al., 1997; Oramas-Wenholz and Quila, 2007). Furthermore, a strong negative correlation between forage production and available protein (AP) ($r = -0.87$), and CP ($r = -0.85$) was noted, which suggest that there may be N dilution effects due to enhanced forage biomass (Belanger and Gastal, 2000; Fletcher and Chakwizira, 2012) in high yielding silage corn genotypes (Figure 3.4.6a &3.4.6b)

4.5.3. Acid detergent fiber (ADF)

Acid detergent fibers (ADF) represent the energetic values of silage corn and comprised of cellulose, lignin and protein, and it is most related to forage digestibility, higher ADF contents, the less digestibility of the forage (Castillo-Jimenez et al., 2009; Moreno-Resendez et al., 2017). Good quality forage crops contains ADF less than 28%, and forage consider to be optimum if ADF contents are less than 30% (Castillo-Jimenez et al., 2009; Gallegos-Ponce et al., 2012; Moreno-Resendez et al., 2017). Manitoba corn committee conducted corn performance trials and evaluate the quality of various hybrids, and found that Yukon R contains 28.7% ADF and Fusion RR 30.5% (Manitoba Corn Committe, 2017). In present study, we found slightly higher ADF in Yukon R 30.5% and 31.5% in Fusion RR. Organic and inorganic fertilization produced 35.9% and 27.2% ADF respectively, in silage corn (Moreno-Resendez et al., 2017). However, DM application produced 28% ADF contents in corn hybrids (Salazar-Sosa et al., 2007). The observed ADF contents in our study varied between 28.06% and 31.58%, (Figure 3.4.4 & Table 3.3), and were in the range as reported by Gallegos-Ponce et al. (2012). P based organic fertilizer applications (e.g., poultry litter and cattle manure) and inorganic P fertilizer produced similar ADF (Nazli et al., 2014). We have also observed similar trend in present study where DM and inorganic P fertilizer had non-significant effects on ADF production in silage corn.

4.5.4. Neutral detergent fiber (NDF)

NDF comprised of hemicellulose, cellulose and lignin representing the fibrous bulk of the forage, and these three components are classified as cell wall or structural carbohydrates and provide the rigidity to plants. NDF in forage crops are negatively correlated with intake and digestibility (Oramas-Wenholz and Quila, 2007), so high energy silage corn hybrids less than 50% NDF are needed (Gallegos-Ponce et al., 2012). Organic fertilization produced 53.5%, whereas inorganic fertilizer produced 42.6% NDF in silage corn forage (Moreno-Resendez et al., 2017). However, combination of inorganic P with poultry litter and cattle manure produce similar forage quality (ADF, NDF and CP) compared to inorganic P fertilization. It appears that NDF contents were significantly reduced with increasing rate of nitrogen fertilizers or nitrogen content in the plant tissue of silage corn (Nazli et al., 2014), which was also reported by Moreno-Resendez et al., (2017). Significant differences in NDF have been observed among the five-tested silage corn genotypes in the present study, for instance, DKC26-28RIB genotype produced 49.60% NDF, which were < 50% and can be considered to be of high quality (Baron et al., 2006; Gallegos-Ponce et al., 2012; Moreno-Resendez et al., 2017). Our results are consistent with the findings reported in the literature (Amodu et al., 2014; Ballard et al., 2001; Baron et al., 2006).

4.5.5. Total digestible nutrients (TDN)

TDN is a measure of feed or forage energy contents, as well as, the forage digestibility in the animals (Posada et al., 2012). Good quality silage contains TDN $\geq$ 65%

(Castillo-Jimenez et al., 2009; Moreno-Resendez et al., 2017; Ramirez et al., 2006). Crop maturity stage significantly influenced the TDN and higher TDN were observed in corn hybrids when harvested at 106–111 days after sowing (DAS) compared to 119–125 DAS (Kim et al., 2001). Manitoba corn committee conducted corn performance trials and evaluated the quality of various hybrids, and found that Yukon R contains 68% TDN and Fusion RR 66.1% (Manitoba Corn Committe, 2017). In present study, we found that Yukon R contains 63% TDN and Fusion RR showed 63.6% TDN. Organic and inorganic fertilizers produced 46% and 47 % TDN respectively in silage corn (Moreno-Resendez et al., 2017). In present study, we found that TDN were slightly higher in manure compared to inorganic P treatment but statistically at par with other P sources, might be due to the initial soil nutrient status, which helped plants to uptake nutrients for growth and TDN (Table 3.4**).**

4.5.6. Net energy for maintenance (NEM) and Net energy for gain (NEG)

Net energy for maintenance (NEM) and net energy for gain (NEG) indicates the quality of forage species (Tine et al., 2001). NEM keep the animals at a stable weight, whereas NEG represents the energy for body weight gain required for maintenance (Schwab et al., 2003). Ballard et al., (2001) reported 1.46 – 1.68 Mcal kg^{-1} NEM and 0.88 – 1.06 Mcal kg^{-1} NEG of dry matter in silage corn hybrids. Manitoba corn committee reported 0.99 NEG Mcal kg^{-1} in Yukon R and 0.94 NEG Mcal kg^{-1} in Fusion RR (Manitoba Corn Committe, 2017). However, in this study, Yukon R contains 0.79 NEG Mcal kg^{-1} and Fusion RR showed 0.81 NEG Mcal kg^{-1}.In present study, we found NEM and NEG of

silage corn genotypes between 1.32 – 1.40 Mcal kg^{-1} and 0.75 – 0.82 Mcal kg^{-1} respectively. This slight decrease in NEM and NEG might be due to harvesting stage, forage fiber contents and lower TDN (Ballard et al., 2001; Di Marco et al., 2002). However, organic and inorganic P sources had non-significant effects on NEM and NEG in silage corn in present study.

4.5.7. Net energy for lactation (NEL)

Energy requirements for maintenance and milk production are expressed as NEL (Moreno-Resendez et al., 2017). NEL is an important energy forage quality parameter estimated using TDN, CP, NDF and other forage quality parameters (Weiss et al., 1992). NEL of silage corn range between 1.3 – 1.5 Mcal kg^{-1} of dry matter as reported in earlier studies (de la Cruz-Lazaro et al., 2007; Nunez-Hernandez et al., 2010; Pena-Ramos et al., 2006). Manitoba corn committee reported that Yukon R contains 1.55 NEL Mcal kg^{-1}, and Fusion RR showed 1.50 NEL Mcal kg^{-1} (Manitoba Corn Committe, 2017). Whereas, in present study, we found that Yukon R showed highest NEL 1.42 Mcal kg^{-1} followed by DKC26-28RIB that produced 1.41 Mcal Kg^{-1} and Fusion RR 1.38 NEL Mcal kg^{-1} (Figure 3.4.3c). Inorganic nutrient source produced significantly higher NEL 1.05 Mcal kg^{-1} compared to organic nutrient source that produced 0.98 Mcal kg^{-1} of NEL (Moreno-Resendez et al., 2017). In present study, NEL were slightly higher under DM with low and high P concentration but statistically at par with inorganic P and control, which might be due to soil ameliorating effects on physiochemical properties of soil (Adeli et al., 2007; Eghball et al., 2004, 2002).

4.5.8. Non-Fibrous Carbohydrates (NFC)

Carbohydrates are classified as fibrous (structural) and non-fibrous (non-structural), as well as the digestibility of fiber affects forage energy (Martinez-Marin, 2008; Moreno-Resendez et al., 2017) The observed NFC in silage corn tested in our experiment were 30.6% – 34.5%, which were quite higher compared to 21% – 31% reported by Moreno-Resendez et al. (2017). Higher NFC contents in silage corn genotypes showed good quality forage. Generally, DM application enhanced NFC contents and in present study, we found higher NFC contents in DM with high P concentration compared to the control (Table 3.4), suggest that DM application could be a sustainable approach to produce forage biomass with higher NFC contents, not only to improve nutrient recycling through DM, but also to minimize the dependency on inorganic fertilizer P due to depleting nature of P reserves (Cordell et al., 2009).

4.6. Conclusion

Results in this study showed that P_1 (dairy manure with high P) significantly improved agronomic performance of silage corn genotypes, soil available P, acid phosphatase activity, soil pH, and microbial communities compared to inorganic P and control. Soil microbial activities were closely linked with organic carbon added through manure application in podzolic soils. Dairy manure (DM) application increased bacterial PLFAs, soil microbial biomass (total amount of PLFAs) and ecological buffering capacity, resulted in higher active G^- bacterial and fungal population relative to control. Pearson correlation also demonstrated a positive and strong relationship between the agronomic

performance parameters of the silage corn genotypes, soil biochemical attributes and active microbial community structure, following DM application with high P. Among the silage corn genotypes evaluated, Yukon R and DKC 26-28RIB showed superior agronomic performance and produced higher DMY compared to other genotypes, whereas, A4177G3RIB produced higher minerals, CP, TDN, NEM, and NEG compared to other genotypes. However, it was statistically at par with DKC26-28RIB in producing minerals and ranked 2nd in protein synthesis. Yukon R was also statistically at par with A4177G3RIB genotype in few other quality indices and produced similar TDN, NEM and NEG. Overall, Yukon–R produced high DMY and was either at par or ranked 2nd in most of the quality forage parameters. Among P sources, inorganic P fertilizer source enhanced CP, simple sugars and NFC contents but statistically at par either with manure with high or low P. Phosphorus sources had non-significant effects on energy parameters, however NEL, NEM and NEG values were slightly higher with DM application compared to inorganic P and control. Taking all together into consideration, it can be concluded that Yukon–R has the potential to be successfully cultivated for attaining higher DMY and high quality forage with DM amendment as P source in podzolic soils under cool climatic conditions of western NL. Considering, additional benefits of DM amendment in enhancing SOC, biochemical attributes and microbial communities suggest DM application can be considered as sustainable agricultural practice and may reduce dependence on inorganic P fertilizers on agricultural lands in future.

4.7. References

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